FINAL REPORT

Development and Use of Genetic Methods for Assessing Aquatic Environmental Condition and Recruitment Dynamics of Native Stream Fishes on Pacific Islands

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14. ABSTRACT

Objectives The overall objective of this project was to develop and demonstrate genetic approaches for assessing the condition (i.e., integrity) of Pacific island watersheds. Traditional assemblage-based protocols provide little basis for discrimination because the biota of Pacific island streams is naturally depauperate and tightly linked to oceanic environments. Genetic assessment protocols can be more effective tools for quantifying watershed condition because genetic analyses can reveal how environmental stressors influence individuals and populations. However, developing and implementing genetic assessment approaches for Pacific island watersheds requires more thorough knowledge of the demographic and ecological processes- especially dispersal and sensitivity to environmental stressors- that give rise to patterns of genetic variation within species that serve as biotic indicators. We undertook a suite of studies focusing on native amphidromous fishes of the Hawaiian Islands to support the development and use of genetic approaches for assessing the integrity of Pacific island watersheds. In the first study, we examined the historical biogeography and contemporary dispersal of native amphidromous fishes across the Hawaiian archipelago. Amphidromy is a form of diadromy, where adults inhabit and spawn in freshwater, larvae drift downstream to near-shore or oceanic environments where they mature for periods of up to six months, after which they recruit back to freshwater streams as postlarvae. The specific objectives of this study were to (1) determine whether different islands harbor distinct evolutionary lineages; and through a multi-disciplinary approach (2) determine whether local recruitment draws from mixed immigrant pools due to larval exchange across the archipelago. This work was intended to help identify the most appropriate spatial scale for management of native fishes and stream ecosystems. In the second study, we assessed among-watershed patterns of genetic variation in relation to local and watershed-scale environmental conditions. This study was intended to illustrate the degree to which key measures of genetic variation (e.g., genetic diversity) within local populations reflect stressor exposure resulting from in-stream habitat degradation or watershed land use patterns. This study was also intended to provide an unprecedented, integrative assessment of watershed integrity across the Hawaiian archipelago. In a related study, we examined how genetic indices vary longitudinally (i.e., from headwaters to the stream mouth) within watersheds. This work was intended to demonstrate whether genetic approaches can discriminate potential effects

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List of Acronyms

AD	– Advection-diffusion
AIC	– Akaike Information Criteria
AIS	– Aquatic invasive species
AMOVA	– Analysis of molecular variance
ANOVA	– Analysis of variance
BMR	– Batch mark-recapture
CB	– Coupled biophysical
CWA	– Clean Water Act
DAR	– State of Hawaii Division of Aquatic Resources
DFA	– Discriminant function analysis
DLNR	– State of Hawaii Department of Land and Natural Resources
DoH	– State of Hawaii Department of Health
DoD	– Department of Defense
ELG	– Early larval growth
ELGD	– Early larval growth anomaly
EPMA	– Electron microprobe
ESA	– Endangered Species Act
FCA	– Factorial correspondence analysis
HYCOM	– 1/25th degree Hawaii HYbrid Coordinate Ocean Models
HWE	– Hardy-Weinberg equilibrium
IBD	– Isolation by distance
IBM	– Individual based model
ICP-MS	– Inductively coupled plasma – mass spectrometer
IMR	– Individual mark-recapture
LA	– Laser ablation
LA-ICP-MS	– Laser ablation inductively coupled plasma mass spectrometry
LD	– Larval duration
LLC	– Limited Liability Company
LLD	– Larval life duration
LLG	– Late larval growth
MANOVA	– Multivariate analysis of variance
MCMC	– Markov-Chain Monte Carlo
MLG	– Mean larval growth
MSA	– Microsatellite Analyser
NAA	– Average number of alleles per locus
NAVFAC	– Naval facilities engineering command
NAWQA	– National Water Quality Assessment Program
NJ	– Neighbor-joining
NHD+	– National Hydrography Dataset Plus
NIST	– National Institute of Standards and Technology
NLCD	– National Land Cover Dataset
NOAA	– National Oceanic and Atmospheric Administration
PACAF	– Pacific air forces
PC	– Principle component factor

PCA – Principle components analysis
PCR – Polymerase chain reaction
PLG – Postlarval growth
PT – Particle-tracking
QDA – Quadratic discriminant analysis
SERDP – Strategic Environmental Research and Development Program
SAP – Special activity permit
SEM – Scanning electron microscope
SIMS – Secondary ion mass spectrometry
SON – Statement of Need
TER-S – Threatened, endangered, and at-risk species
TL – Total length
USAF – United States Air Force
US EPA – United States Environmental Protection Agency
USGS – United States Geological Survey

List of Abbreviations

‰ – Per mil
%ag-urb – Percent agricultural and urban land cover
2D – Two-dimensional
3D – Three-dimensional
A_r – Allelic richness
Ba – Barium
bp – Base pair
C – Carbon
^oC – Centigrade degree
Ca – Calcium
Cps – Counts
Cr – Chromium
Cs – Cesium
Cu – Copper
cm – Centimeter
CO1 – Cytochrome Oxidase I
CV – Coefficient of variance
cytb – Cytochrome b
DNA – Deoxyribonucleic acid
dNTP – Deoxyribonucleotide
F – Forward
f – Apparent recruitment
FW – Freshwater
GM – Geometric mean
H – Hydrogen
HCl – Hydrochloric acid
H_e – Expected heterozygosity

k – The number of populations
K – von Bertalanffy growth curve
KYA – Thousand years ago
L – Liter
L∞ – Theoretical asymptotic maximum length
La – Lanthanum
µl – Microliter
µm – Micrometer
m – Meter
m_1 – First generation migrant
mg – Milligram
Mg – Magnesium
$MgCl_2$ – Magnesium chloride
min – Minutes
ml – Milliliter
mm – Millimeter
mM – Millimolar
Mn – Manganese
mtDNA – Mitochondrial DNA
MYA – Million years ago
ηg – Nanogram
N_e / N – Ratio of effective population size to census population size
N_e – Effective population size
N – Nitrogen
N_2 – Atmospheric nitrogen
Neb – Effective population size estimate
N-hat – Population size for each capture event
NH_4 – Ammonium
Ni – Nickel
NO_3 – Nitrate
O – Oxygen
OH – Hydroxide
p – Capture probability
P – Phosphorus
Pb – Lead
pent – Probability for entry into the population
phi – Survival
ppm – Parts per million
PO_4 – Phosphate
R – Reverse
Rb – Rubium
s – Seconds
SD – Standard deviation
SE – Standard error
Sr – Strontium
SPR – Species richness

SRP – soluble reactive phosphorus
SW – Marine
T – Trophic position
TDN – Total dissolved nitrogen
TSS – Total suspended sediment
TDS – Total dissolved solids
TP – Total phosphorus
U – Unit
V – Vanadium
VIE – Visible implant elastomer tags
wt% – weight percent
Zn – Zinc

Keywords

Pacific islands; Hawaii; Oahu; aquatic environmental assessment; water quality; watershed management; ocean-stream connectivity; amphidromy; goby; *Awaous stamineus*; *Sicyopterus stimpsoni*; historical biogeography; genetic diversity; genetic differentiation; population connectivity; population density; immigration; species diversity; environmental impairment; invasive species; Poeciliids; watershed land use; impervious surfaces; water chemistry; nutrient loading; mitochondrial DNA; microsatellite markers; mark-recapture; snorkel survey; otolith microchemistry; coupled biophysical modeling

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1 Abstract

Objectives
The overall objective of this project was to develop and demonstrate genetic approaches for assessing the condition (i.e., integrity) of Pacific island watersheds. Traditional assemblage-based protocols provide little basis for discrimination because the biota of Pacific island streams is naturally depauperate and tightly linked to oceanic environments. Genetic assessment protocols can be more effective tools for quantifying watershed condition because genetic analyses can reveal how environmental stressors influence individuals and populations. However, developing and implementing genetic assessment approaches for Pacific island watersheds requires more thorough knowledge of the demographic and ecological processes- especially dispersal and sensitivity to environmental stressors- that give rise to patterns of genetic variation within species that serve as biotic indicators.

We undertook a suite of studies focusing on native amphidromous fishes of the Hawaiian Islands to support the development and use of genetic approaches for assessing the integrity of Pacific island watersheds. In the first study, we examined the historical biogeography and contemporary dispersal of native amphidromous fishes across the Hawaiian archipelago. Amphidromy is a form of diadromy, where adults inhabit and spawn in freshwater, larvae drift downstream to near-shore or oceanic environments where they mature for periods of up to six months, after which they recruit back to freshwater streams as postlarvae. The specific objectives of this study were to (1) determine whether different islands harbor distinct evolutionary lineages; and through a multi-disciplinary approach (2) determine whether local recruitment draws from mixed immigrant pools due to larval exchange across the archipelago. This work was intended to help identify the most appropriate spatial scale for management of native fishes and stream ecosystems. In the second study, we assessed among-watershed patterns of genetic variation in relation to local and watershed-scale environmental conditions. This study was intended to illustrate the degree to which key measures of genetic variation (e.g., genetic diversity) within local populations reflect stressor exposure resulting from in-stream habitat degradation or watershed land use patterns. This study was also intended to provide an unprecedented, integrative assessment of watershed integrity across the Hawaiian archipelago. In a related study, we examined how genetic indices vary longitudinally (i.e., from headwaters to the stream mouth) within watersheds. This work was intended to demonstrate whether genetic approaches can discriminate potential effects arising from activities on Department of Defense (DoD) installations from effects arising from natural processes or other anthropogenic activities elsewhere in the same watershed.

Technical Approach
In the first study, we examined within- and among-island patterns of mitochondrial deoxyribonucleic acid (mtDNA) haplotype frequencies in *Awaous stamineus* and *Sicyopterus stimpsoni* to infer the presence of island-specific evolutionary lineages and geographic patterns of historical colonization of the archipelago. To assess contemporary dispersal, we coupled an existing archipelago-wide model of ocean circulation to a particle-tracking model to determine the probability that larvae entering the ocean would re-enter their natal stream, streams on the same island, or streams on different islands over two years assuming a short or long larval duration. We tested model predictions by examining nuclear microsatellite genotype frequencies in both species. We also reconstructed movement potential and life history variation of *A.*

stamineus from otolith microchemistry, which involved examining standard isotopic ratios, novel oxygen isotope ratios, and panels of trace elements. In the second study, we assessed watershed-scale patterns of covariance among environmental variables and measures of genetic diversity, population densities, and species diversity. This involved comparison of mtDNA and microsatellite-based estimates of genetic variation to native and non-native species population densities, assemblage structure, water chemistry, and watershed land use. Similar comparisons were conducted to assess relationships across longitudinal transects within four watersheds. To support both studies, estimates of population densities derived from snorkel surveys were validated through comparison to estimates derived from mark-recapture approaches.

Results

The two studies generated a rich portfolio of findings, including the following:

- ***A. guamensis in Hawaii is a distinct species (A. stamineus) relative to Indo-Pacific congeners***
- ***Neither A. stamineus nor S. stimpsoni exhibit island-specific evolutionary lineages across the Hawaiian archipelago; however, neither species exhibits panmictic population structure***
- ***A. stamineus exhibits a facultative rather than an obligatory amphidromous life history***
- ***A large proportion of A. stamineus larvae remain in or near their natal stream and ocean-going larvae occupy both near-shore and off-shore marine habitats***
- ***Genetic diversity of A. stamineus and S. stimpsoni declines with increasing densities of invasive species and watershed conditions related to nutrient loading***
- ***Population densities and genetic diversity of native fishes in watersheds that host military activity are comparable to levels in forest and ag-urban dominated watersheds on Oahu***
- ***Population densities of native fishes are depressed in watersheds across Oahu, regardless of land use or stewardship***

These and additional findings yielded the following conclusions:

- ***Immigrant pools may draw from populations across the archipelago, and the influx of postlarvae may sustain local populations in degraded waterways, but among-island dispersal cannot sustain at-risk populations as it has little influence on local population dynamics.***
- ***Site-specific and watershed-scale conditions may supersede the importance of physiographic conditions in structuring genetic, demographic and assemblage variation of native amphidromous species in oceanic island streams.***
- ***Aggregate effects can arise from local and watershed-scale degradation, where the cumulative influence of biotic or abiotic stressors can disrupt processes that promote the persistence of native fishes across entire islands.***

Benefits

This research has increased fundamental knowledge of insular stream ecology and responses of native amphidromous fishes to environmental stressors. By demonstrating the extent to which core biological measures of watershed integrity reflect local degradation and the loss of connectivity between streams and adjoining near-shore habitats, these studies also show that integrative protocols tailored to capture estimates of genetic variation alongside measures of population density and species diversity can provide a robust basis for watershed management on oceanic islands. Accordingly, information gained from this research will better enable DoD resource managers and collaborators to maintain habitat for at-risk aquatic species, thereby further ensuring the sustainability of military training activities and guiding the restoration of degraded streams across the Pacific.

2 Objectives

Department of Defense (DoD) activities can give rise to anthropogenic stressors that threaten freshwater stream ecosystems on oceanic islands across the Pacific. The sustainability of DoD activities in these locations may be jeopardized if services provided by insular freshwater stream ecosystems are not effectively managed or restored. Accomplishing the goal of effective stream and watershed management will require improved understanding of the ecology of insular freshwater stream ecosystems, their responses to anthropogenic stressors, and interactions with near-shore marine environments. To improve overall management and restoration efforts to sustain DoD activities, the Strategic Environmental Research and Development Program (SERDP) issued Statements of Need (SON) for research that leads to (1) the acquisition of representative information on the ecology of freshwater streams on Pacific islands and potential interactions with near-shore marine ecosystems; (2) the development of ecological indicators for military training impacts on stream ecosystems and watersheds; (3) identification and quantification of the effects of terrestrial non-native, invasive species control activities on freshwater streams, watersheds and associated near-shore ecosystems; (4) the development of restoration methods; and (5) the development of novel invasive species control techniques to assist restoration efforts. The research described herein addresses SON #1 and #2.

The overall objectives of this project were to obtain essential information on amphidromous fishes of the Hawaiian Islands (SON #1) to enable use of genetic methods for assessing the integrity of oceanic island watersheds (SON #2). Patterns of genetic variation in fishes bear signatures of population persistence and colonization relative to in-stream conditions (Waits et al. 2008) and watershed land use (Bagley et al. 2004, Blum et al. 2005). Patterns of genetic variation may also reflect connectivity and dispersal geometry across multiple spatial scales (Waits et al. 2008, Blum et al. 2012). Genetic diversity, genetic subdivision and effective population size of native fishes are therefore core metrics of genetic protocols for assessing watershed integrity. However, developing and implementing genetic assessment approaches for Pacific island watersheds requires thorough knowledge of the demographic and ecological processes that give rise to patterns of genetic variation within native amphidromous fishes.

To address SON #1, a study was carried out to supply information on key historical and contemporary demographic processes to better understand patterns of genetic variation in native amphidromous fishes across the Hawaiian Islands. The specific objectives of this work were to:

- Study objective 1.1: Reconstruct phylogeographic patterns of historical colonization across the archipelago with reference to other Indo-Pacific archipelagos
- Study objective 1.2: Infer movement potential and ocean-stream connectivity across the archipelago according to coupled biophysical modeling, population genetic analysis, and analysis of otolith microchemistry

This work was motivated by the hypothesis that species of native fishes do not exhibit island specific evolutionary lineages and exhibit panmictic population structure because of marine larval dispersal across the archipelago. By testing this hypothesis, this work aimed to identify the most appropriate spatial scale for management of native fishes and oceanic island stream ecosystems. Under panmictic conditions, for example, it is possible that locally at-risk

populations may be 'rescued' or sustained by larval immigration from populations elsewhere in the archipelago. Thus, extirpation of at-risk populations may be less likely if immigrant pools draw from sources across the archipelago. Accordingly, DoD-led management of stream ecosystems should consider that local populations are susceptible to local impacts as well as impacts elsewhere in the archipelago that affect areas that serve as net sources of migrants. If immigrant pools are not mixed, but instead comprise individuals derived exclusively from local populations, then resident populations of native fishes may be more susceptible to within-basin or within-island impacts than is now thought.

To address SON #2, a study was carried out to supply information on the sensitivity of genetic indicators (e.g., genetic diversity) and traditional biotic indicators (e.g., species diversity) to in-stream and watershed-scale environmental conditions. The objectives of this work were to:

- Study objective 2.1: Characterize land cover across a set of watersheds representing relatively undisturbed forest cover, agricultural-urban land use, and military stewardship on all five of the Hawaiian Islands (Hawaii, Maui, Molokai, Oahu, Kauai) that support perennial streams
- Study objective 2.2: Characterize a panel of water chemistry parameters across the studied watersheds
- Study objective 2.3: Characterize genetic variation of two focal species as well as the assemblage and community structure of native and non-native aquatic species across the studied watersheds
- Study objective 2.4: Characterize and calibrate population densities and demographic parameters of native amphidromous fish that serve as core biological indicators
- Study objective 2.5: Examine covariance between genetic indicators and traditional biotic indicators, as well as biotic and abiotic in-stream conditions, and land use within watersheds, among watersheds on each island, and among islands.

This study was carried out to determine whether genetic measures serve as sensitive indicators of (1) site-level and or/watershed-scale degradation; and (2) the susceptibility of at-risk species to abiotic and biotic stressors (e.g., invasive species, contaminants). If sensitivity tracks in-stream habitat degradation and not watershed land use, for example, then maintaining the integrity of riparian zones and improving in-stream conditions could prove most beneficial to native biota. If sensitivity tracks land use (and/or in-stream conditions linked to land use), reducing impacts derived from conditions across broader spatial scales (e.g., non-point source pollution) would likely be more beneficial. The study also was intended to test the hypothesis that immigration permits local populations to endure under degraded or isolated conditions. If so, then inland populations of native fishes are likely to be demographic sinks and persistence is likely greatest at sites closest to putative sources of colonization. By providing detailed information on susceptibility and population persistence, it was expected that this work would demonstrate the utility of genetic methods for assessing the structure, functioning, and integrity of oceanic island watersheds. In turn, this would provide DoD managers with a widely-applicable new tool for prioritizing management, restoration and conservation priorities.

3 Background

3.0 Oceanic Island Watersheds and Stream Ecosystems

Department of Defense (DoD) resource managers are in need of improved watershed assessment protocols to support management and restoration efforts intended to mitigate DoD activities that threaten freshwater stream ecosystems on Pacific islands. Current assessment protocols for Pacific island watersheds rely on measures of native fish assemblages to rate levels of stream impairment. These protocols are likely to underestimate impairment because native fish assemblages of insular streams are naturally depauperate. Because nearly all native stream fishes on Pacific islands are amphidromous (amphidromy is a migratory life history where adults inhabit and spawn in freshwater, larvae drift downstream to near-shore or oceanic environments where they mature for periods of up to six months, after which they recruit back to freshwater streams as postlarvae), responses to environmental stressors may also be misunderstood if consideration is not given to the processes that sustain populations in degraded waterways and near-shore habitats. Advancing Pacific island watershed management and restoration therefore requires development of indicators that are responsive to the ecology of insular freshwater stream ecosystems, including interactions with near-shore marine environments.

Assessment and monitoring protocols designed for Pacific island freshwater stream ecosystems (e.g., Kido 2002) are much like those designed for continental stream ecosystems (e.g., Lazorchak et al. 1998). However, freshwater stream ecosystems on oceanic islands exhibit characteristics that limit the applicability of assessment protocols derived from protocols designed for continental systems. Watersheds on oceanic islands, such as the Hawaiian Islands, are comparatively small and narrow by continental standards (Kinzie 1988, Brasher 2003, Smith et al. 2003). As a consequence, stream length is shorter and channel geomorphology is relatively straight and steep (Smith et al. 2003, Craig 2003). Flow regimes of insular streams, especially those on low-latitude islands, have been characterized as being “flashy” due to rapid responses to periodic and often intense precipitation (Smith et al. 2003, Covich et al. 2003, Craig 2003, Oki and Brasher 2003). Because insular streams are event-driven, their discharge rates and flow velocities are highly variable relative to continental systems (Resh and de Szalay 1995).

Insular stream ecosystems also differ from continental systems because habitat formation and availability shift during the course of island geomorphic development. Freshwater habitats across a lineament of hotspot volcanic islands, like the Hawaiian Islands, reflect geomorphic changes predominantly driven by erosion (Craig 2003). Newly formed islands may not support systems with permanent running water if rainwater percolates through porous volcanic material. As an island ages, channels eventually form after vegetation becomes established and soils develop. Subsequent formation of deeply incised valleys can be rapid, although the rate of incision depends on the composition of underlying volcanic material and lava viscosity. As catchment area increases, stream networks and specialized microhabitats— such as grottos, stair-step cascades, and seaward terminus waterfalls— develop due to variations in volcanic material or the succession of riparian vegetation (Craig 2003).

Along with island area and isolation (MacArthur and Wilson 1963), the successional development and heterogeneity of aquatic habitat in insular stream ecosystems contribute to the

evolution and biogeography of freshwater stream biota. Because the evolution of oceanic island stream biota is contingent on the successional state of streams, the balance of speciation and extinction of stream biota within an island may differ from broad principles of island biogeography, including species-area relationships (Smith et al. 2003, Craig et al. 2001, Craig 2003). For example, species richness of stream assemblages can be higher on a comparatively older, but smaller island with greater habitat heterogeneity than a larger, younger island (Donaldson and Myers 2002). In addition, species richness of oceanic island stream assemblages may not be a function of isolation because physiological or life history traits that enable initial colonization may also limit the potential for intra-island speciation and adaptive radiations (MacArthur and Wilson 1963, Zink et al. 1996, Chubb et al. 1998, Fitzsimons et al. 2002, McDowall 2003). This is most evident in the Hawaiian Islands, which support a freshwater fish fauna consisting of only five native species, all of which have amphidromous life histories.

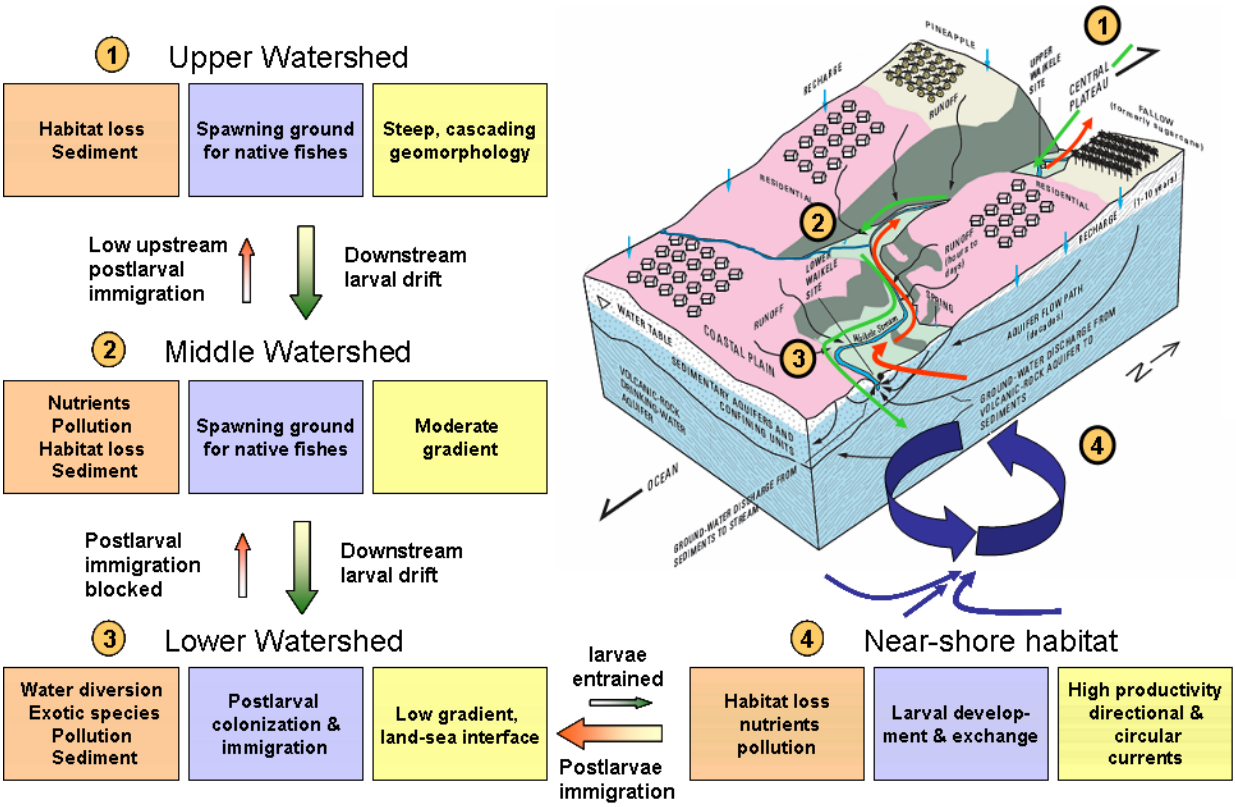

Figure 1: Conceptual model of an oceanic island stream ecosystem and interface with near-shore habitat. Example given is of a mixed-land use watershed with specific reference to the influence of urbanization, agriculture, and water diversions on native amphidromous fishes. Base map of Waikele Stream on Oahu redrawn from Anthony et al. (2004). Boxes in orange identify biotic and abiotic stressors, boxes in blue reference demographic processes, and boxes in yellow reference characteristic physical or geomorphological attributes particular to the specified location in the watershed.

Conceptual models of stream ecosystems on oceanic islands therefore must account for the importance of marine habitats as a dispersal corridor linking stream ecosystems, as well as geomorphology and human activity in the context of island biogeography. Figure 1 provides an overview of how physical characteristics, biological processes, and conservation threats vary across elevational zones of an oceanic island stream ecosystem. This conceptual model illustrates the diversity of factors that must be incorporated into strategies for watershed management and

restoration on oceanic islands. It also demonstrates that concepts derived from studies of continental watersheds cannot necessarily be applied to manage or restore oceanic island watersheds. Given the growing pressures that are being placed on freshwater resources and services (e.g., drinking water, water for irrigation, fishery resources, habitat for at-risk or threaten species), sustaining the integrity of oceanic island stream ecosystems requires substantive improvement of current watershed management and restoration approaches. On islands where past or ongoing military activities co-occur with other anthropogenic pressures on freshwater resources (e.g., diversions for irrigation), the challenges of conserving biodiversity and sustaining oceanic island stream ecosystems can be particularly acute. Ensuring that demands can be met without the loss of at-risk species requires improving current watershed management approaches. A critical step toward this goal is the use of assessment protocols specifically designed for oceanic island streams.

The Hawaiian Islands offer opportunities to examine the influence of anthropogenic impacts on native stream communities (Brasher 2003) and to test the effectiveness of novel assessment protocols relative to existing protocols. The Hawaiian Islands serve as a model for understanding oceanic island geomorphic development (Carson and Clague 1995), ecosystem functioning (Vitousek 2004), and island biogeography (e.g., Gruner 2007). As a result, Hawaiian freshwater stream ecosystems have been studied more intensively than streams on other oceanic islands (Resh and de Szalay 1995, Craig 2003, Brasher 2003). The native species assemblages of Hawaiian stream ecosystems also are unusually depauperate even in comparison to other Pacific island archipelagos (Donaldson and Myers 2002, Fitzsimons et al. 2002, McDowall 2003). Moreover, the five native stream fishes are at great risk of extinction due to urbanization, water diversions, and military activities that contribute to habitat degradation or increase contaminant loads in streams.

The five stream fishes native to the Hawaiian Islands are members of the goby family (Gobiidae, including Eleotridae following Hoese and Gill (1993)). All exhibit an amphidromous life history. Amphidromy is a form of diadromy, where adults inhabit and spawn in freshwater, larvae drift downstream to near-shore or oceanic environments where they mature for periods of up to six months, after which they recruit back to freshwater streams as postlarvae. The fauna consists of *Awaous stamineus* (Valenciennes), *Lentipes concolor* (Gill), *Eleotris sandwicensis* Vaillant and Sauvage, *Sicyopterus stimpsoni* (Gill), and *Stenogobius hawaiiensis* Watson (McDowall 2003). All are Hawaiian endemics with the possibly exception of *A. stamineus*, which may occur elsewhere in the Indo-Pacific (Lindstrom et al. 2012). This species, which was once referred to as *A. guamensis*, was thought to also occurs on Guam in the Mariana Islands, New Caledonia, Vanuatu and Fiji (Watson 1992), but recent work has shown that populations in the Hawaiian archipelago are genetically distinct from populations that occur in the Mariana Islands (Lindstrom et al. 2012). In the past, several species have been considered at-risk or threatened (Devick et al. 1995), but no species is presently listed or being considered for listing as threatened or endangered. However, given their status as species of special concern, the presence and relative abundance of native fishes are currently used as key indicators of stream integrity for the purposes of watershed management.

3.1 Genetic Assessment of Aquatic Environmental Condition

Like other federal agencies that steward public lands, the DoD is subject to the Clean Water Act (CWA) and the Endangered Species Act (ESA). The agency is therefore accountable for the integrity of surface waters and critical habitat in oceanic island watersheds that fall within its stewardship portfolio. More robust methods for stream monitoring and assessment could better enable the DoD to meet CWA and ESA regulatory compliance requirements.

Many protocols currently used to assess oceanic island streams employ potentially uninformative or insensitive measures of condition. For example, protocols implemented in the Hawaiian Islands (Burr 2001, Kido 2002, Henderson 2003) are modifications of protocols for continental stream ecosystems that involve measuring the diversity and relative abundance of native fishes to rate the condition of stream segments. Though these measures reflect the conservation importance of Hawaiian stream biota, they offer limited guidance for management and restoration of insular stream ecosystems. The information content of ratings based on assemblage-level characteristics, such as the loss of native species diversity or the presence of native species tolerant to water quality impairment or habitat degradation, can be high in continental streams with speciose assemblages, but it is minimal in the Hawaiian Islands because the native fish assemblage is naturally depauperate (Parham 2005). Thus, current protocols that rely on assemblage-level measures offer little basis for discriminating between levels of impairment, and could even bias assessments towards underestimating impairment.

Because all native stream fishes in the Hawaiian Islands are amphidromous, responses to environmental stressors may also be misunderstood if consideration is not given to the processes that sustain populations in degraded waterways (Waits et al. 2008). The persistence of local populations of amphidromous fauna likely depends upon conditions in both streams and in adjoining near-shore habitats. For instance, adult fish in a stream may fail to reproduce, but the population could persist if sustained by immigration of marine-dispersing postlarvae originating from other streams. If a population within a stream is sustained by continuous upstream immigration from near-shore environments, severe site impairment would be masked by condition ratings based on assemblage-level data (Lazorchak et al. 1998, Burr 2001, Kido 2002, Henderson 2003). Protocols that are tailored to reflect insular stream ecology, including the importance of interactions with near-shore marine ecosystems, could offer a stronger basis for management and restoration of oceanic island streams and conservation of constituent biota.

Genetic protocols are well suited for assessing insular stream ecosystems because they are not constrained by low species diversity and because they can be used to characterize processes that sustain species in degraded conditions (Bagley et al. 2004, Blum et al. 2005, Schwartz et al. 2007). Patterns of genetic variation bear signatures of population persistence and colonization relative to in-stream conditions (Waits et al. 2008) and watershed land use (Bagley et al. 2004, Blum et al. 2005). Geographic patterns of genetic variation may also reflect connectivity and dispersal geometry at multiple spatial scales (Waits et al. 2008, Blum et al. 2012). For example, habitat modifications that reduce passage of immigrants and restrict gene flow can increase genetic drift within subdivided populations and divergence among populations (Pannell and Charlesworth 1999, Hébert et al. 2000). Exposure to environmental stressors, such as organochlorine pesticides, can also select against intolerant genotypes and consequently alter the

size, viability, and genetic diversity of populations (McMillan et al. 2006, Waits et al. 2008). By providing estimates of population persistence and by characterizing responses to environmental stressors, genetic assessment of insular stream biota holds great promise for improving watershed management. Similarly, by identifying the processes that promote recovery of native species to anthropogenic impacts, genetic assessment of insular streams can potentially improve efforts to restore critical habitat (Bagley et al. 2004, Waits et al. 2008, Blum et al. 2012).

Genetic approaches have already been utilized to assess environmental condition in streams with low species diversity (Waits et al. 2008), and to evaluate the viability of species exhibiting a range of life histories, including migratory life histories comparable to amphidromy (Àlo and Turner 2005). With some modifications, existing approaches can be used to assess impairment of oceanic island streams and to examine native species' responses to environmental stressors. However, modification and implementation of genetic assessment protocols requires empirical studies that detail the genetic structure of focal species across multiple spatial scales and in relation to location-dependent and hierarchical environmental heterogeneity (Fagan 2002, Blum et al. 2012). Improving basic knowledge of population dynamics (i.e., movement potential) and native biota responses to environmental stressors also can enable greater inferential power and afford more precise application of genetic approaches.

3.2 Historical Colonization and Contemporary Connectivity

Degradation of in-stream and near-shore conditions can potentially lead to complete loss of local populations. Prior studies suggest, however, that local populations of native stream fishes might be rescued by immigration from populations elsewhere in the Hawaiian archipelago. For instance, within-archipelago gene flow appears to be high in all five native species, and no evidence has been found that island-specific evolutionary lineages occur within any of the species (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998). A parsimonious explanation for the absence of island endemics is that frequent dispersal among islands within the archipelago has prevented local differentiation. Zink et al. (1996) proposed that dispersal within the archipelago is driven by amphidromy, where stochastic recruitment draws from well-mixed pools of immigrating postlarvae.

The inferred absence of within-archipelago diversification due to larval exchange among islands has important management implications. If immigrant pools are mixed and populations are panmictic, then management of stream ecosystems must account for the susceptibility of local populations of native fishes to both local impacts and to impacts elsewhere in the archipelago that serve as net sources of immigrant postlarvae. Unlike in continental stream ecosystems, where local populations largely recruit from immigrants originating from elsewhere within a watershed (e.g., Waits et al. 2008, Lamphere and Blum 2012), the scale of metapopulation dynamics of oceanic island stream fishes may span whole island archipelagos. Thus, reconstitution of a locally extirpated population would require both robust populations elsewhere in the archipelago and favorable stream conditions for maturation and reproduction in a restored stream segment. On the other hand, if native amphidromous species exhibit within-archipelago population subdivision and if postlarvae frequently return to their natal stream, then resident populations of native stream fishes may be more susceptible to within-basin or within-island

impacts than is now thought. Local populations would be more prone to extirpation because within-basin or within-island impacts could diminish adult populations and locally derived off-shore immigrant pools. In this case, maintenance or restoration of local in-stream and near-shore habitats should be prioritized by management efforts aiming to sustain local populations of native amphidromous fishes.

Several attempts have been made to characterize archipelago-wide patterns of genetic variation within each of the gobies native to the Hawaiian Islands (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998), but prior to the work presented here, a comprehensive analysis involving sufficiently informative molecular markers and exhaustive sampling had yet to be conducted. Moreover, no study had directly assessed movement potential and contemporary population connectivity. Inferences about the absence of population subdivision and the mixed composition of immigrant pools largely reflect two studies (Zink et al. 1996, Chubb et al. 1998) that found evidence of high mitochondrial DNA (mtDNA) haplotype diversity within each species and an absence of monophyletic groups of haplotypes corresponding to different islands. Both of these findings have been viewed as evidence that local populations recruit from mixed immigrant pools and that source-sink dynamics are regional rather than local due to larval exchange among islands. However, the results of these studies are far from conclusive because data were collected from small numbers of specimens. Zink et al. (1996) examined an average of 23 individuals per species (< 5 individuals per species per island), while Chubb et al. (1998) only examined an average of 14 specimens per taxon (< 3 individuals per species per island). With the advent of less expensive, high-throughput molecular techniques, subsequent studies of other island taxa have shown that stronger inferences of population structure can be derived from geographic differences in mtDNA haplotype frequencies (e.g., Jordan et al. 2005) or multi-locus genotype frequencies (Walter et al. 2011). Recent findings indicating that local retention of pelagic larvae and natal homing are stronger among marine dispersing fishes than has been previously thought (Thorrold et al. 2001, Taylor and Hellberg 2003, Pampoulie et al. 2004, Sorenson et al. 2005) further underscore the need for completing comprehensive genetic studies of Hawaiian gobies.

As a necessary first step towards the development and use of genetic protocols for assessing oceanic island watersheds, we re-examined the hypotheses that (1) native amphidromous fishes do not exhibit island-specific evolutionary lineages; and that (2) recruitment draws from mixed immigrant pools due to larval exchange among islands. Through complementary genetic analyses, analyses of otolith microchemistry, and simulation modeling, we examined alternative hypotheses of differentiation and recruitment dynamics (Figure 2), including (1) panmixia where recruitment draws from a well-mixed immigrant pool (2) directional isolation-by-distance attributable to island geomorphic development and recruitment from a well-mixed immigrant pool (3) non-directional isolation-by-distance attributable to within-archipelago post-colonization dispersal from an initial stochastic founding event and recruitment from a mixed immigrant pool (4) island-specific allopatric differentiation reflective of island geomorphic development and recruitment from a predominantly local immigrant pool and (5) island-specific allopatric differentiation reflective of recruitment from a local immigrant pool but not of island geomorphic development.

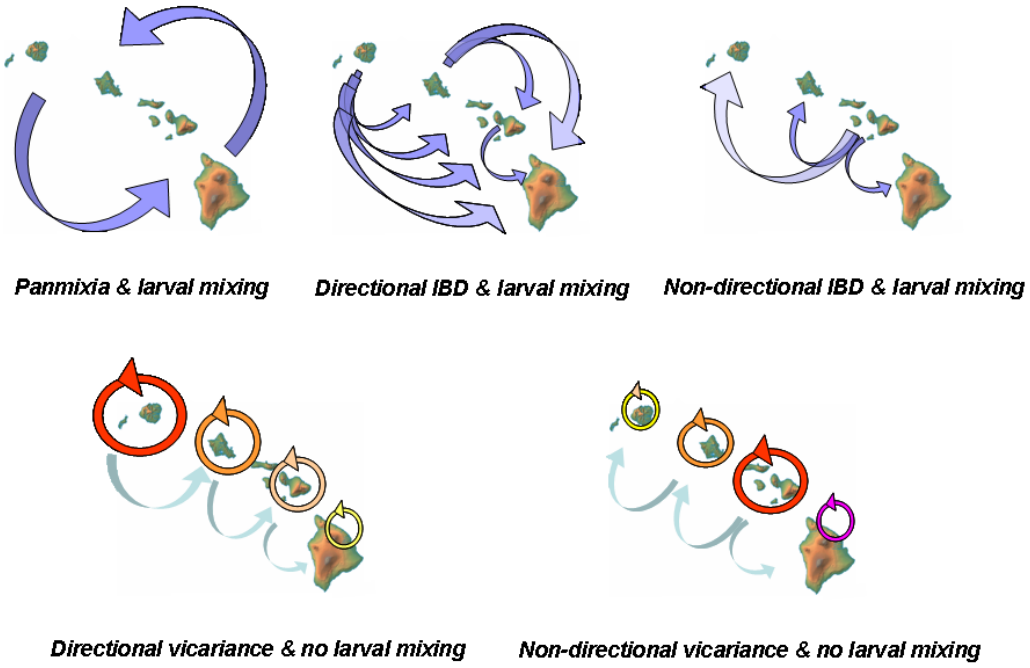

Figure 2: Hypotheses of geographic structure and larval recruitment of native amphidromous fishes in the Hawaiian Islands. From top left to right: (1) panmixia and well-mixed immigrant pools, (2) directional isolation-by-distance (IBD) where the strength of gene flow diminishes from older to younger islands and immigrant pools are mixed, (3) directional isolation-by-distance where the strength of gene flow diminishes from an ancestral founder population and immigrant pools are mixed. From bottom left to right: (4) directional vicariance where younger islands are sequentially seeded by propagules from older islands and immigrant pools are local, and (5) non-directional vicariance where islands are sequentially seeded following a stochastic founder event and immigrant pools are local.

3.2.1 Genetic Analysis of Historical Colonization and Contemporary Connectivity

We examined the population genetic structure of two native Hawaiian amphidromous fishes to test alternative hypotheses of colonization and contemporary connectivity. We compared within- and among-island patterns of genetic variation exhibited by *S. stimpsoni*, an endemic, moderately intolerant species capable of dispersing far inland, to patterns exhibited by *A. stamineus*, a putatively endemic, but possibly more cosmopolitan (Lindstrom et al. 2012) tolerant species common to stream reaches at lower and middle elevations (Figure 3). For each species, we characterized both mtDNA haplotype frequencies and nuclear microsatellite allelic differentiation to assess geographic patterns of genetic variation in order to reconstruct patterns of historical colonization and to assess whether recruitment draws from mixed immigrant pools due to larval exchange among islands.

Examining geographic patterns of genetic variation within *S. stimpsoni* and *A. stamineus* required collecting tissue samples of adults from each of the high islands where the species are known to occur. Where possible, specimens of *A. stamineus* and *S. stimpsoni* postlarvae were also collected. Analysis of adult specimens provided a basis for assessing geographic patterns of differentiation related to historical colonization of the archipelago. Comparisons of adults also

afforded a basis for estimating contemporary dispersal, and comparison of adults to immigrating postlarvae provided a basis for assessing whether immigrant pools are derived from local or regional sources.

Figure 2: The research presented here focuses on two species: (left) *Awaous stamineus* (photo by Keoki Stender) and (right) *Sicyopterus stimpsoni* (photo by M. Yamamoto and A Tagawa).

3.2.2 Use of Otolith Microchemistry for Estimating Contemporary Connectivity

To complement our examination of spatial patterns in genetic variation, we also inferred individual movement potential from the chemical composition of otoliths (ear stones) obtained from *A. stamineus*. Otoliths offer an ontogenetic counterpart to the phylogenetic information encoded in genetic data. As otoliths are formed, their calcium-carbonate matrix passively incorporates many trace elements from the water– including Strontium (Sr), Barium (Ba), Magnesium (Mg), Manganese (Mn), and Lead (Pb)– creating a multivariate chemical fingerprint. The relative concentrations of these elements, and in some cases their isotopic composition (e.g., ^{87}Sr:^{86}Sr), can vary widely among aquatic ecosystems as a function of local geochemistry and anthropogenic contamination. This variation is recorded in the otoliths, making it possible to distinguish fish from different locations. Moreover, otolith material is laid down continuously as fish grow, thereby creating a detailed, permanent record of the chemical environment experienced from the larval stage to the time of sampling. The combination of sensitive genetic and chemical methods not only can yield a more definitive characterization of the spatial scale of dispersal in at-risk, native fishes of the Hawaiian Islands, but it also can yield unexpected discoveries of life history variation and life-long consequences of alternative dispersal strategies.

Natural variation in elemental composition is expected among Hawaiian streams due to differences in age among islands and sharp gradients in mineral weathering by precipitation within islands (Vitousek 2004). These differences should be augmented by localized anthropogenic contamination from waste water and combustion vapors (Sutherland 2000, de Carlo et al. 2004), and by military activities that generate Pb and Ba contamination (Ashwood and Olsen 1988, USAF 2000). Previous analyses of Hawaiian stream fish have demonstrated clear Strontium:Calcium (Sr:Ca) signatures of larval movement from high-Sr marine habitats into low-Sr streams (Figure 4), and ontogenetic profiles of movement have indicated likely differences among individuals within and between populations (Radtke and Kinzie 1996, Benson and Fitzsimmons 2002). In addition, estimates of larval life duration from otolith rings have shown differences among islands that may reflect differences in currents (Radtke et al. 2001). Analyses of trace elements other than Sr have not yet been attempted with Hawaiian freshwater fish, but the utility of trace element and isotopic fingerprinting for distinguishing stocks has been

proven (Swearer et al. 1999) in anadromous salmon and marine fish at scales of tens of kilometers (km).

We have analyzed geographic variation in otolith microchemistry of *A. stamineus* from the same sites surveyed for genetic variation within and among islands. This broad geographical sampling was used to map gross patterns of trace element abundance in streams across the archipelago. We also analysed otolith transect profiles to reconstruct the life history and movement potential of individuals sampled from across the archipelago. Each transect crossed the otolith core, which is formed during early larval development in the natal stream. Each transect then ran through sections of the otolith corresponding to periods of larval dispersal, and terminated at the otolith edge, which corresponds to the stream habitat where individuals were captured. Our intention

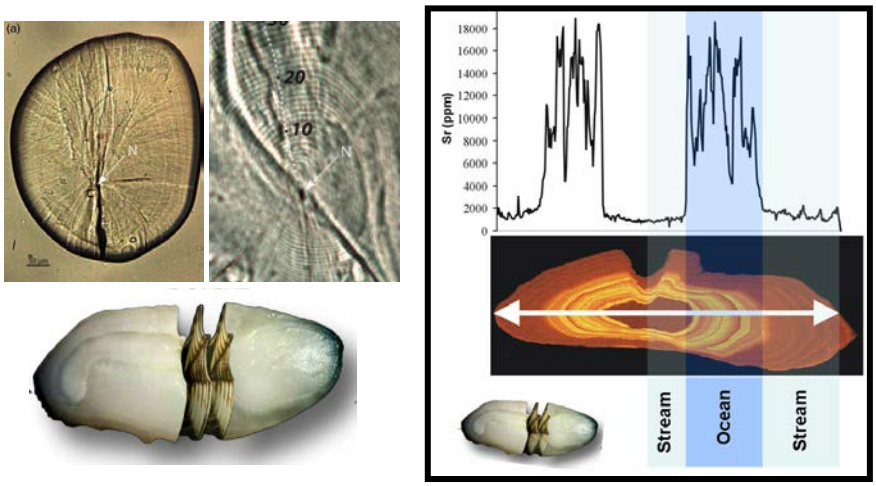

Figure 4: Example of (left, top left and bottom) an otolith cross-section and (left, top right) resolution of daily growth rings; Example (right) of a transect analysis of an otolith cross section indicating that the individual began its life in a low-strontium freshwater environment, migrated through a high-strontium oceanic environment, and then returned to a freshwater environment .

was to evaluate the proportion of individuals with matching natal and adult stream signatures to generate an index of natal retention versus population mixing. This was based on the expectation that, if trace element mapping finds predictable patterns, then the chemical nature of mismatches between natal and adult streams can elucidate the population sources contributing to migration within and among islands. However, due to the difficulty of consistently identifying the otolith core (which only represents ≤2 days of development and growth in the natal stream), we shifted our focus to evaluating the conditions associated with periods of larval dispersal.

To obtain further information on individual-level responses to natural variation and anthropogenic stressors, we examined individual growth rates and the length of the larval period of *A. stamineus* using visual assessments of otolith microstructure. These data allow inferences to be drawn about correlates of individual- and population-level patterns, thereby maximizing the value of in-depth profiles of fish densities, in-stream conditions, and watershed land cover for streams across the Hawaiian archipelago.

Measuring individual growth rates can be a sensitive assay of responses to in-stream and watershed conditions. Previous research has demonstrated that Hawaiian gobies exhibit daily growth rings in otoliths (Radtke et al. 1988). In combination with length or mass data, ring counts allow precise estimation of growth rates of individuals as well as representative growth trajectories for a sample site. We analyzed the growth rate of every *A. stamineus* for which

otoliths were sampled. We also estimated growth trajectories over all individuals collected at a given site. Mean individual growth rates and growth trajectories were then regressed against summary measures of in-stream and watershed conditions to better understand the mechanisms underlying human impacts on individual fish and local populations across the archipelago.

The length of the larval period, which can be a fundamental driver of dispersal potential, is known to vary among islands and species in Hawaiian gobies (Radke et al. 1988, Radtke et al. 2001). To better characterize patterns of variation, we also measured larval life duration (LLD) for every *A. stamineus* for which otoliths were available. By counting the daily rings lying inside a dark band known as the "settlement mark," which is thought to indicate return to freshwater (though it appears to better corresponds to metamorphosis; Hogan et al. 2012), LLD was compared among individuals, sites, and islands. To evaluate the causes of differential LLD, we compared larval period length to larval habitat use, as inferred from individual otolith microchemistry during the larval period. This enabled us to test the hypothesis that fish using the open ocean have longer larval periods than those that remain inshore in coastal marine, estuarine, or freshwater environments. To evaluate population-level consequences of differences in larval period, we regressed mean larval period against the proportion of dispersing individuals inferred from otolith trace elements. This enabled us to determine whether populations with long mean larval periods are more likely to disperse between islands than those with short larval periods.

3.2.3 Use of Oxygen Isotopes in Otoliths for Reconstructing Life History

As the only lifelong record of the environment experienced by a fish, otoliths offer a unique compliment to genetic data for reconstructing individual movement. Several sources of variation in environmental chemistry are recorded in otoliths. As noted above, otoliths record chemical differences that occur between freshwater and marine environments, which can provide information on amphidromy in Hawaiian gobies (Radtke et al. 1988, 1996). At the watershed level, differential age and weathering of similar parent materials (Vitousek 2004; Figure 4) is expected to be recorded in otoliths, yielding island-specific and potentially watershed-specific signatures. Finally, urban catchments in the archipelago may exhibit abnormally high levels of certain trace elements (Sutherland 2000, de Carlo et al. 2004), and military activities have been associated with elevated lead (Ashwood and Olsen 1988) and barium (USAF 2000; Figure 4). Both watershed-specific signatures and signatures of anthropogenic activity can provide information on life-stage and life-long responses to prevailing environmental conditions.

The application of tools such as laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has allowed for retrospective analyses of habitat use, movement and life-history parameters from time-stratified variation in otolith microchemistry (Elsdon et al. 2008). Such inferences are generally based on patterns in minor and trace elements such as Sr, Ba, Mg and Mn that are rare yet consistently present within the calcium carbonate matrix of otoliths (Campana 1999). Secondary ion mass spectrometry (SIMS) is a promising new tool for elucidating environmental history and fish movement between habitats based on otolith microchemistry. One benefit of SIMS compared with LA-ICP-MS is higher precision of the sampling beam (Durrant and Ward 2005), which leads to finer temporal resolution. In fast-growing fish, SIMS can allow sampling of each individual daily growth increment (Weidel et al.

2011). An additional benefit of SIMS is the ability to precisely quantify stable isotope ratios of Carbon (C) and Oxygen (O), which is not possible using LA-ICP-MS.

Isotope-specific fractionation of O is based on a predictable deterministic relationship with ambient water temperature, where ^{18}O is slightly enriched in warmer waters due to preferential evaporation of the lighter ^{16}O (Thorrold et al. 1997). Prior work has shown that records of $\delta^{18}O$ (the ratio of ^{18}O to ^{16}O) variation can offer temperature resolution of 1 C° and temporal resolution of roughly two weeks (Hoie et al. 2004, Weidel et al. 2007). However, preferential evaporation of ^{16}O also means that rainwater is depleted of ^{18}O and so freshwater systems, if driven largely by rainwater, will be depleted compared to seawater. Thus, $\delta^{18}O$ variation may be a proxy for environmental temperature and/or salinity conditions experienced by a fish.

Few studies have taken advantage of the power to merge microstructural and microchemical analyses of otoliths to investigate early life history, even though such integrated analyses can substantially increase inferential power (Hamilton et al. 2008, Shima and Swearer 2009, Hogan et al. in review). For example, integrative approaches can resolve growth rings corresponding to the larval period by both chemical and visual means. In collaboration with geochemists at the University of Wisconsin, we used a CAMECA-IMS-1280 ion microprobe to achieve high-precision measurements of $\delta^{18}O$ at 10 µm spatial resolution following coating with a thin film of gold (Weidel et al. 2007), with microprobe targeting guided by light microscope and scanning electron microscope (SEM) imagery. Examining $\delta^{18}O$ variation afforded us novel perspectives for interpreting challenging trace element and isotopic variation profiles examined during the course of our work on watershed-scale assessments (see section 3.2.3). This involved combining three different techniques (daily otolith increment analysis, LA-ICP-MS and SIMS) for the first time, where novel data on $\delta^{18}O$ variation was captured from otoliths that were also analyzed for trace elements, other isotopes, and microstructural variation. Thus, this work offered the potential to achieve novel methodological and ecological insights. For instance, we occasionally observed large swings in trace element concentrations even in individuals that resided in a specific stream reach. Data on $\delta^{18}O$ variation could help elucidate whether these shifts are attributable to groundwater input dynamics or other factors, thereby clarifying the interpretation of trace element data. This approach also enabled us to examine variation in the early life history of *A. stamineus* with the objective of inferring habitat use of individual larvae and investigating the consequences of differential habitat use on growth performance. Though salinity differences between freshwater and marine environments can be profound, and therefore readily detectable through methods involving LA-ICP-MS analyses of otolith microchemistry, salinity does not necessarily differ strongly between near-shore and estuarine habitats. However, our survey data (see sections 3.3.1 and 3.3.2) showed that stream water temperatures are cold in comparison to marine waters; 80% of streams across the archipelago are ≤25°C, and almost half are ≤23°C. In contrast, National Oceanic and Atmospheric Administration (NOAA) sea surface data indicate that nearshore marine waters are generally 26-28°C, while offshore waters are 25-26°C (http://www.nodc.noaa.gov/dsdt/cwtg/ hawaii.html). These temperature disparities are theoretically sufficient to distinguish whether the larval period—which is identifiable in each otolith using trace element variation and visual assessment of settlement mark (following Radtke et al. 1988)—was spent in fresh, estuarine, nearshore marine, or offshore marine waters. By providing a new source of information on the likelihood and potential outcomes of local retention, this study also greatly enhanced our capacity to interpret genetic data on dispersal.

3.2.4 Coupled Biophysical Modeling of Larval Dispersal

Biophysical modeling serves as a complementary method alongside genetic and otolith-based approaches for addressing questions about conditions that promote local retention and the probability of long distance dispersal. By affording opportunities to assess basic assumptions of larval transport, biophysical modeling can provide a predictive framework for determining the amount of variation in connectivity that is attributable to passive (ie. ocean circulation, long distance transport) and active dispersal (ie. vertical migration, local retention). This in turn can advance understanding of how species responses to in-stream and watershed stressors (natural, military or other anthropogenic factors) depend on interactions with near-shore marine environments, and therefore improve the use and power of genetic indicators of aquatic environmental condition.

Oceanographic modeling of marine-dispersing larvae often assesses outcomes of passive dispersal through advection and diffusion (Okubo 1994). Advection-diffusion (AD) models simulate transport of particles (i.e. larvae) in the direction of mean water flow (advection), and the process of transport of particles by random motion from an area of high concentration to low concentration (diffusion). AD models are typically two-dimensional (2D) representations of surface currents and treat marine larvae as passive particles (Black 1988). AD models of 2D passive dispersal tend to overestimate larval exchange, especially over longer distances (Cowen et al. 2000), and therefore may over-estimate larval dispersal distances. Empirical studies of larval transport among islands have reinforced this perspective, repeatedly showing evidence of higher local retention than expected from AD models of 2D passive dispersal (Swearer et al. 1999, Jones et al. 1999, Jones et al. 2005).

Greater understanding of larval biology and the advent of greater computing power have resulted in coupled biophysical (CB) models, a class of oceanographic models that consider the ocean matrix in three dimensions (3D) and that can be joined with biological models of larval behavior, life cycles and life history. CB models can account for the possibility that larvae do not behave passively, but instead exhibit behaviors that promote local retention or that alter dispersal trajectories (Kingsford et al. 2002). In comparison to AD models, CB models have proven to be significantly more powerful predictors of connectivity and larval dispersal among islands and across coastlines (e.g., Cowen et al. 2006, Kobayashi 2006).

Coupled biophysical models of larval dispersal are comprised of two to three separate models where the output from one model informs or drives another. CB models of larval dispersal rely on a 3D ocean circulation model to track the direction and velocity of currents in a given region of interest, such as the Hawaiian Islands (Kobayashi 2006). Current information from the ocean circulation model is then input into an individual-based particle-tracking algorithm (PT algorithm) that calculates the movement of simulated particles (i.e., larvae) through 3D ocean space. The algorithm uses real-time outputs from the ocean circulation model to determine the movement of individual particles in successive time-steps from a user-defined release point (ie. spawning site) until a user-defined length of time has elapsed. At the end of the run-time the position of each “larva” is recorded. A wide range of biological parameters can be included in the PT algorithm including larval life duration, vertical migration behavior, swimming ability,

and sensory ability. Inclusion of realistic biological parameters and parameter values that describe particles in PT algorithms tend to improve CB model predictions of dispersal and recruitment (Paris and Cowen 2004, Kobayashi 2006).

We undertook CB model simulations to evaluate larval transport according to spawning location, ocean circulation and physical forcing across the Hawaiian Islands. Our study objectives were to (1) characterize the potential distances and pathways over which demographically meaningful quantities of larvae might be expected to disperse under passive advection-diffusion conditions; (2) evaluate spatial variability in passive dispersal outcomes; and (3) compare the outcomes of passive dispersal for genetic and otolith-based data on connectivity to infer the likelihood of active (i.e., vertical migration and individual fitness differentials) dispersal of goby larvae across the Hawaiian Islands.

Though similar objectives have been addressed in prior modeling studies of larval transport in the central Pacific and elsewhere (Paris et al. 2005, Kobayashi 2006, Paris et al. 2007), larval transport has been poorly characterized among stream-bearing islands in the Hawaiian archipelago and larval transport of amphidromous species has never been modeled. As in other studies of similar intent (Cowen et al. 2003, Paris et al. 2005, Kobayashi 2006), we modeled larval transport using a combination of an available high-resolution ocean circulation model and individual-based modeling techniques. This enabled us to examine spatially explicit individual-based modeling scenarios intended to replicate larval transport during production, settlement and recruitment. This also enabled us to simulate larval transport across space and time to address how outcomes vary according to productivity, location, seasonal, and inter-annual events that might influence ocean circulation (e.g., El Niño conditions). Model outputs from alternative scenarios were first qualitatively summarized and compared as heuristic maps. Outcomes of alternative scenarios also were quantitatively assessed as connectivity matrices to (1) characterize the probability of larvae arriving to each watershed from all possible source populations, and (2) define the relative contribution of recruits from local retention versus long distance dispersal events. Doing so served to clarify inferences of population connectivity from observed patterns of genetic variation and otolith microchemistry.

By providing the first descriptions of amphidromous larval transport, this study addressed one of the most important (yet also one of the most poorly understood) processes that can sustain at-risk populations of species native to oceanic island stream ecosystems that are often highly degraded or intensively managed for water resources. Biophysical modeling of larval transport can also provide a framework for partitioning variation in connectivity across the full life history of amphidromous species. Because modeling larval transport can help characterize the outcomes of ongoing and planned watershed management and restoration projects on DoD installations, it can serve as a valuable forecasting tool for assessing the potential benefits of individual or coordinated management actions to population connectivity among watersheds across Hawaii. For example, the model we have developed could help (1) establish the likelihood of preventing the collapse of a local population; (2) determine potential sources for the recovery of a local population; or (3) determine the timeframe for natural recolonization of a restored stream. Being able to assess these and other relevant scenarios could help DoD managers allocate resources to more effectively maintain or enhance habitat for at-risk species.

3.3 Genetic and Integrative Assessment of Pacific Island Watersheds

There is abundant evidence that degraded stream and riparian habitat conditions arising from land use and water diversions have negative effects on stream fishes native to the Hawaiian Islands (Brasher 2003). As adults of these species all reproduce in freshwater stream habitat (Ego 1956, Ha and Kinzie 1996), stream alterations such as channelization can eliminate spawning habitat and alter the diet of native fishes (Brasher 2003). Newly hatched larvae drifting downstream toward the ocean also can be entrained in surface water diversions (Brasher 2003). Similarly, upstream immigration of juvenile postlarvae returning to freshwater can be blocked by in-stream barriers (Benstead et al. 1999) or eliminated when streams are fully dewatered (Brasher 2003). The longitudinal distribution of adults in streams may also shift in response to barriers and dewatering (Brasher 2003). Stream alterations and dewatering can have even stronger negative consequences if conditions favor non-native fishes that prey upon, compete with, or spread parasites to native fishes (Font 2003).

Less is known about how in-stream degradation, dewatering and land use impact near-shore habitats. Degraded stream conditions and land use can increase sedimentation and nutrient loading of near-shore habitats, especially in estuarine areas sheltered from off-shore currents. It is possible that such conditions could be lethal to larvae of native fishes that develop as plankton in near-shore habitat over periods of weeks to months (McDowall 2003). Stream diversions could also sever species' life cycles by eliminating environmental cues that enable postlarvae to locate streams (Kinzie 1988) or that direct upstream migration (Smith and Smith 1998). Loss of immigration cues could increase the stochasticity of postlarval recruitment, reduce larval survivorship and increase the risk of local extirpation (Kinzie 1988), which highlights the importance of using assessment protocols that account for oceanic island streams interfacing with near-shore marine habitats.

3.3.1 Among-Watershed Assessment of Environmental Condition

We have undertaken a study to test the hypothesis that the well-being and integrity of local populations- measured in terms of genetic diversity, population size and immigration- varies according to stressor exposure resulting from in-stream habitat degradation and/or watershed land use patterns. To evaluate the effects of environmental stressors on populations of native Hawaiian stream fishes, we have compared genetic, population-level, and community-level indices to stream habitat conditions and watershed land use. To examine stressor response and indicator performance, we adopted a balanced hierarchical sampling design to account for within-watershed, between-watershed, between-island, and temporal variance across the following three land use categories: military (watersheds used for military exercises or that are dominated by a DoD installation), forested, or mixed agricultural-urban (ag-urban; watersheds that are dominated by agricultural and urban land cover). Under ideal conditions, this approach would have involved comparing data from three replicate reaches within three replicate watersheds of each land use category on each of three islands (i.e., 81 sites across 27 watersheds), with repeated sampling to obtain robust estimates of genetic structure, effective population size (herein defined according to Wright (1931) as the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele

frequencies under random genetic drift or the same amount of inbreeding as the population under consideration) and immigration, and population size / density (Waples 1989, Nielsen et al. 1999, Turner et al. 2002). We modified this design to include sets of forested and ag-urban sites on two additional islands, and to reflect the clustering of military watersheds on Oahu. Though several military installations are located on other islands, none occur in watersheds with perennial streams. The implemented design thus expanded the scope of among-watershed comparisons, and among-island comparisons of forested and ag-urban watersheds. Clustering of military watersheds on Oahu, however, prevented statistically valid comparisons of military land use across islands.

We examined native fish responses to alteration of landscape and in-stream conditions through genes-to-ecosystems comparisons. This involved collecting tissues or full post-metamorphic specimens of *A. stamineus* and *S. stimpsoni* at all sites for genotyping at species-specific panels of nuclear microsatellite markers. Where possible, specimens of *A. stamineus* and *S. stimpsoni* postlarvae were also collected. Building from the results of the study of archipelago-wide patterns of genetic variation (see section 3.2.1), we also obtained mtDNA sequence data for all tissues and specimens of *A. stamineus*. Population densities of all native macrofauna (i.e., fish, molluscs, and shrimp) were also surveyed at each location, and fish assemblage structure, in-stream habitat conditions, and water chemistry were characterized following standard protocols (Lazorchak et al. 1998, Kido 2002). Land use within each watershed was characterized from satellite imagery (Blum et al. 2005, Blum et al. 2012), and nitrogen stable isotope ratios of algae, macroinvertebrates (i.e., gastropods) and *A. stamineus* were used as a time-integrated measure of anthropogenic nutrient loading (Fry 1999, Schlacher 2005). The sensitivity of genetic, population and assemblage-level indices to environmental stressors was evaluated through regression models incorporating measures of in-stream and landscape conditions (Blum et al. 2012). We also examined pair-wise relationships to better understand the mechanistic basis of variation in biotic indices relative to environmental heterogeneity.

3.3.2 Within-Watershed Assessment of Environmental Condition

The condition (i.e., integrity) of military watersheds might not be solely attributable to effects arising from military activities. Understanding the influence of military stewardship or activities on water quality or biotic condition may call for differentiating effects arising from DoD installations from natural variation and/or effects from other human activity within the same watershed. Regionally depressed productivity (i.e., island-wide effects) and correspondingly low local retention of marine-dispersing larvae could be contributing factors. Effects arising from natural processes or non-military anthropogenic activities within military watersheds (i.e. upstream or downstream from a DoD installation) may also be confounding factors. On densely populated islands, such as Oahu, DoD installations may be surrounded by a host of other entities, ranging from intensive agricultural operations to mixed residential-commercial urban neighborhoods. Attributing impacts to military activity rather than other proximate factors thus requires use of assessment approaches that are capable of discriminating reach-scale (i.e., longitudinal) variation in stream condition.

Naturally depauperate species diversity and longitudinal variation in assemblage-level characteristics complicate use of traditional ecological indicators for assessing and comparing

the condition of sites within watersheds on oceanic islands. The naturally low number of co-occurring species in oceanic island streams declines even further from lower to higher elevation stream reaches. Upstream colonization requires the ability to climb (sometimes vertical surfaces) and climbing ability varies among native amphidromous species, so the distribution of species within watersheds differs according to climbing ability (Figure 5). In the Hawaiian Islands, for example, all five native amphidromous fishes can occupy stream reaches below the first major cascade or waterfall, whereas *Lentipes concolor*, which has fully fused pelvic fins and strong fin musculature, is the only species capable of colonizing headwater reaches above the highest waterfalls (Figure 5). Consequently, the information content of ratings based on assemblage-level indicators also declines while the possibility of underestimating impairment increases. Changes in information content may also be irregular because shifts in species diversity and distributions within watersheds can be abrupt and step-wise (rather than continuous) relative to the height and longitudinal position of in-stream barriers. Longitudinal distributions in streams may also shift in response to dewatering (Brasher 2003). Therefore longitudinal shifts likely differ among watersheds, and possibly among islands if the nature of in-stream barriers reflects island age in archipelagos (as in the Hawaiian Islands) where geomorphic changes are driven by erosion (Craig 2003).

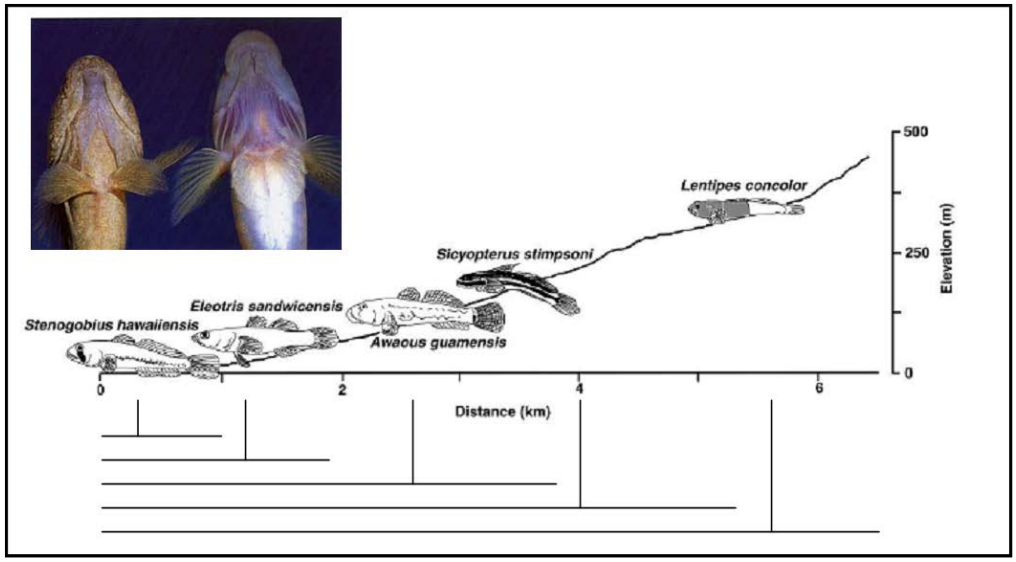

Figure 5: Longitudinal distribution of native amphidromous species in a Hawaiian stream. Overlap depends on climbing ability, which varies among species according to fin musculature. Inset: Unfused pelvic fins of *Eleotris sandwicensis* (left) and fused pelvic fins of *Awaous stamineus* (right), referred to as *Awaous guamensis* in the diagram (modified from Evenhuis and Fitzsimons 2007).

In contrast, genetic indicators of aquatic environmental condition– including core metrics like genetic diversity, genetic subdivision and effective population size of native species– are well suited for assessing and comparing the condition of sites within watersheds on oceanic islands. Genetic indicators are not constrained by low species diversity or longitudinal variation in assemblage-level structure because genetic indicators can reflect stream condition relative to ocean-stream connectivity and dispersal geometry within and among watersheds (e.g., Blum et al. 2005, Waits et al. 2008, Lamphere and Blum 2012). Patterns of genetic variation also can bear signatures of population persistence and colonization relative to in-stream conditions (Waits

et al. 2008) and watershed land use (Bagley et al. 2004, Blum et al. 2005, Blum et al. 2012). Thus patterns of genetic variation can reveal how environmental stressors affect individuals and populations (Schwartz et al. 2007) within spatially explicit frameworks (i.e., river-stream networks within watersheds).

To extend our study of variation among watersheds throughout the Hawaiian archipelago**,** we have undertaken a complementary study to examine longitudinal patterns within watersheds to better differentiate effects arising from natural conditions and anthropogenic activity. Though the approach taken for assessing among-watershed variation (i.e., sampling three replicate sites per watershed) provided data on among-site variation, it is not sufficient for resolving within-watershed, longitudinal variation in stream condition. To provide more detailed understanding of within-watershed patterns of variation, we have characterized longitudinal patterns of (1) genetic diversity, effective population size and immigration; (2) mark-recapture estimates of population size, condition and individual movement; (3) snorkel survey estimates of population densities and assemblage-structure; and (4) site-specific water chemistry and habitat conditions. This enabled us to provide longitudinal characterization of within-watershed patterns of stream condition according to (1) local in-stream characteristics; and (2) relationships between local in-stream characteristics (ie. distance over which genetic diversity recovers downstream of an impacted site); with reference to (1) watershed-scale land use; and (2) island-wide degradation.

Additionally, this work enabled us to compare effective population to census populations size (e.g., N_e /N), which offers perspectives on reproductive evenness in a population and provides a time-integrated index of population persistence and resiliency (Frankham 1995, Palstra and Ruzzante 2008). We examined local effective population size, as determined from genetic data, relative to census population size, as determined from visual surveys and/or mark-recapture data. This enabled us to assess whether taxa exhibit strongly nonrandom mating patterns, such as "sweepstakes" breeding, where a small subset of individuals produce the majority of offspring (Hauser and Carvalho 2008). It also allowed us to infer whether anthropogenic stressors reduce reproduction but not adult survival (Theodorakis 2003, Theodorakis et al. 2006).

Longitudinal sampling has been carried out within four watersheds. We examined a military dominated watershed on Oahu, and to exclude potential effects arising from island-wide degradation, we also examined longitudinal variation in a forest dominated watershed and two ag-urban dominated watersheds on Hawaii. Longitudinal transects of sites were examined within each watershed from lower reaches (1 meter (m) to 20 m elevation), middle reaches (20 m to 200 m elevation, and above the first 10 m barrier but below the first 20 m high barrier), and to upper reaches (above 200 m or above the first 20 m barrier but less than 750 m) as defined by the Hawaii Division of Aquatic Resources (DAR). The location of each site varied according to accessibility, notable in-stream features such as diversions, and notable watershed land use features such as DoD installations. This approach enabled us to test the following hypotheses:

H1: **Populations of native fishes located further inland and at higher elevations are demographic sinks because the likelihood of persistence decreases with distance from sources of immigrating postlarvae (demographic sink hypothesis)**

Expected patterns: Genetic diversity, effective population size and immigration will decline from coastal to inland sites. No directional shifts in genotypes will occur. Age-size structure of populations will skew towards larger and older individuals.

H2: **Populations of native fishes located further inland and at higher elevations are a genetic subset of coastal and lower elevation populations due to adaptive differences in climbing ability and predator avoidance (selective sieve hypothesis)**

Expected patterns: Effective population size and immigration will decline from coastal to inland sites. No change in genetic diversity will occur, and a directional shift in genotypes will occur. Census population sizes will decline, but age-size structure of populations will not be skewed.

H3: **Variation among populations of native fishes located from lower coastal sites to higher elevation inland sites corresponds to site-specific conditions (local impacts hypothesis)**

Expected patterns: Changes in genetic diversity, effective population size and immigration will be idiosyncratic to site-specific conditions. Similarly, genotypic composition and age-size structure of populations will reflect site-specific conditions.

The hypothesized scenarios are not necessarily mutually exclusive. It is possible, for example, that site-specific and steady declines in genetic diversity may occur, indicating that longitudinal variation reflects both natural processes and local impacts.

Though detailed characterization of within-watershed variation is, by itself, essential for understanding stressor responses and indicator performance, coupling this knowledge with additional information gained from other complementary studies can further advance understanding of how species responses to in-stream and watershed stressors correspond to interactions with near-shore marine environments. This can in turn provide more powerful assessment, monitoring (and potentially forecasting) tools for DoD management of oceanic island watersheds.

3.3.3 Mark-recapture Calibration of Snorkel Surveys

Development of innovative approaches that more directly reflect insular stream ecology can improve management and restoration of oceanic island stream ecosystems, so long as consideration is given to attendant societal perspectives and public discourse. Native amphidromous fishes and invertebrates are important cultural resources in the Hawaiian Islands. Although none of the amphidromous species are currently listed as threatened, endangered or of concern, the heritage value of each species affords them a status that confers some protection by the State of Hawaii. Historically, species were abundant enough to support an artisanal fishery, but population declines due to anthropogenic impacts (Brasher et al. 2006, Walter et al. 2012) have led to a fishing ban (Ha and Kinzie 1996). State protection extends to minimizing destructive sampling for research through oversight and review of special activity permits (SAPs). In addition to reducing take by emphasizing non-lethal sampling, the Hawaii DAR has established moratoriums on collection methods that may be potentially harmful or injurious to

native amphidromous fauna. Consequently, research intended to improve assessment protocols must involve approaches that reflect protective controls set by the permit approval process.

Achieving nearly all of our key research objectives required efficient methods for capturing fish and quantitative methods for estimating census population size of the targeted species and stream fish assemblage structure (i.e., the composition, diversity and relative abundance of stream fish species) at each sample site. Characterizing assemblage structure and census population size provides a basis for inferring the spatial distribution of potential demographic sinks within and among watersheds across the archipelago due to time-integrated effects of abiotic and biotic (i.e., invasive species) stressors on recruitment of native fishes. Capturing specimens also enables comparisons of genetic protocols to current assessment protocols. We originally intended to use modified Breder traps (Benbow et al. 2004), minnow seines, and electrofishing to capture fish for genetic and otolith analysis. We also planned to characterize assemblage structure and to estimate census population sizes by depletion electrofishing.

Though electrofishing has become the foremost capture method for collecting fish (Snyder 2003, Murphy and Willis 1996) for stream assessment work across North America and elsewhere, the Hawaii DAR currently does not permit use of electrofishing for research or other purposes. Electrofishing provides the greatest return for the least effort among stream fish capture methods, making it especially useful for detecting rare species (Poos et al. 2007) and assessing assemblage structure (Murphy and Willis 1996). When applied using repeat-pass depletion methods, electrofishing represents the only nonlethal capture method that allows census population size to be quantitatively estimated in a single site visit, making it the most efficient estimation method for sampling many sites over broad spatial scales (Sutherland 2006, Murphy and Willis 1996). Yet electrofishing is a practice that runs contrary to cultural mores in Hawaii, and it could unnecessarily exacerbate other risk factors by possibly injuring fish (Snyder 2003). The effectiveness of electrofishing for benthic fish has also been questioned, particularly in streams with restrictive habitat (Baker and Foster 1992). The prevalence of plunge pools and boulder substrates in Hawaiian streams can restrict application of electrofishing, and all Hawaiian gobies lack swim bladders as adults, which can substantially reduce electrofishing capture efficiency (Polacik et al. 2008). Despite these concerns, research on a comparable assemblage of amphidromous gobies in Puerto Rico has demonstrated that depletion electrofishing generates reliable population size estimates (Kwak and Cooney 2008).

Following a review by the State of Hawaii Board of Land and Natural Resources, we received SAPs with negotiated limits on non-lethal and lethal take and protective conditions for capturing fishes. Neither SAP permitted electrofishing. This challenged us to implement alternative capture methods (i.e., hand netting) and to estimate population size and assemblage structure via visual snorkel surveys- the survey method promoted by the DAR. The permit conditions motivated us to implement a precedent-setting calibration study to ensure that we were obtaining high quality data on census population sizes of Hawaiian gobies. Our objective in doing so was to support DAR policy and public sentiment, and to enable broader comparison of survey results to findings of demographic studies done elsewhere.

Visual surveying can produce high quality population estimates, but site- and habitat-specific characteristics, especially stream size and turbidity, may bias population estimates (Thompson

2003). The standard method for estimating abundance and distributions of stream fish in Hawaii is a point-quadrat visual survey approach (Higashi and Nishimoto 2007) that generates more quantitative estimates of fish density than transect methods (Baker and Foster 1992). The point-quadrat method that has been widely implemented in Hawaii involves snorkelers conducting a visual census of resident fish at random points within a stream. Census counts are made at 30 points within the reach to attain a representative sample, and counts are assumed to be exhaustive. At each point, fish are counted within an observation area of no more than 1 m by 1 m, an area selected to avoid missing fish, particularly those in smaller size classes (Baker and Foster 1992). Population size estimates from visual surveying often correlate well with estimates from depletion electrofishing (Thompson 2003). Calibration using an independent method of estimating population size (Hankin and Reeves 1988) can correct for potential biases, resulting in population estimates that are equivalent or superior to those derived from other survey approaches (Sutherland 2006, Carrier et al. 2009).

Mark-recapture approaches are, arguably, the best calibration option for visual surveying in Hawaii because the method can result in accurate population estimates even if capture probability is relatively low (Rosenberger and Duhnam 2005). In exchange for the added time investment for hand-netting and marking fish (i.e., approximately five minutes per fish) and a return site visit for recapture, the method can offer a more accurate and precise estimate of population size with fewer fish captures compared to depletion methods that can exhibit biases associated with low capture probabilities (Peterson et al. 2004, Carrier et al. 2009).

We undertook a mark-recapture study to calibrate the standard point-quadrat method of surveying fish populations in Hawaiian streams. We conducted both individual and batch mark-recapture studies following preliminary exercises intended to optimize marking and recapture methods. Batch mark-recapture sampling with a single recapture event was carried out to provide site-level information on local population sizes across longitudinal transects within the four watersheds where within-watershed assessments were carried out (see section 3.3.2). We conducted a more intensive individual mark-recapture study, involving three or more recapture events over a full year, at a subset of sites within three of the four watersheds. This was intended to provide site- and period- specific estimates of individual growth, movement, and survival of adults as well as recruitment of postlarvae to resident populations. Batch mark-recapture sampling with a single recapture event was also carried out at sample sites in a subset of the watersheds included in the archipelago-wide study of watershed condition (see section 3.3.1).

Together, this work was intended to generate (1) high quality estimates of adult abundances, and (2) longitudinal estimates of adult growth, survival, as well as recruitment to adult populations in watersheds of differing land use and in-stream conditions. To identify physical and biological factors influencing demographic conditions, we also evaluated relationships between estimates of abundance with abiotic and biotic measures within and among watersheds. This was intended to allow for comparisons to genetic and otolith microchemistry data in order to strengthen inferences about the spatial scales of demographic variability.

Initially, we also intended to produce watershed-level and longitudinal estimates of reproductive productivity and recruitment. In amphidromous taxa, reproduction and recruitment can be directly assessed via the abundance of emigrating larvae and returning postlarvae. Between June

and November 2010, we attempted to quantify drifting larvae and returning postlarvae in each of our three study watersheds on Hawaii. Techniques for collecting emigrating larvae, detailed in Lindstrom et al. (1999), proved to be too inefficient to produce reliable density estimates. Even though sampling for drifting larvae was regularly conducted at each of our mark-recapture sites, capture rates were consistently near zero. This may have been related to severe drought conditions that persisted across the Hawaiian archipelago throughout 2010 (i.e., the Hilo airport recorded only 50% of normal annual rainfall in 2010, which registered as the driest year in the past 30 years). Lindstrom (1998) hypothesized that drought conditions can limit the reproductive output of adult gobies. Following Benbow et al. (2004) and Burky et al. (2005), postlarvae were collected during the spring of 2010 near study stream mouths using modified Breder traps. Though the traps captured small numbers of returning postlarvae, the technique did not provide a representative sample of postlarval density. Traps rarely captured more than one postlarva, even at sites where visual surveys documented significantly larger numbers (i.e., hundreds) of returning postlarvae. Thus, more efficient capture methods are needed to accurately assess larval export and postlarval re-entry in Hawaiian streams.

4 Materials and Methods

4.0 Historical Colonization and Contemporary Connectivity

To test the hypothesis that native amphidromous fishes do not exhibit island-specific evolutionary lineages, we employed genetic approaches to reconstruct patterns of historical colonization of two exemplar species across the Hawaiian archipelago. We tested the hypothesis that recruitment draws from mixed immigrant pools due to larval exchange among islands by examining patterns of genetic variation and otolith microchemistry relative to coupled biophysical model simulations of larval dispersal.

4.0.1 Genetic Analysis of Historical Colonization and Contemporary Connectivity

Study species: We compared within- and among-island patterns of genetic variation exhibited by *Sicyopterus stimpsoni*, an endemic, moderately intolerant species capable of dispersing far inland, to patterns exhibited by *Awaous stamineus*, which is a more tolerant (putatively) endemic species common to stream reaches at lower- and middle-elevations on each island (Keith 2003, Lindstrom et al. 2012). For each species, we characterized both mitochondrial DNA haplotype frequencies and nuclear microsatellite allelic differentiation to assess geographic patterns of genetic variation.

Site locations, sampling design, and sample collections: To estimate geographic patterns of genetic variation, tissue samples were collected from *S. stimpsoni* and *A. stamineus* from streams on Kauai, Maui, Molokai, and Hawaii. Only *A. stamineus* was captured on Oahu. Permit conditions prevented us from capturing *S. stimpsoni* on Oahu because it is now rare on the island (Burr 2001, Henderson 2003, Blum et al., unpublished data, Hawaii Watershed Atlas; http://hawaii.gov/dlnr/dar). Neither species is known to occur on Niihau, Lanai, or Kahoolawe, although exploratory surveys suggest that native stream fauna may occur in headwater refugia on Lanai. In accordance with historical collection records (Hawaii Watershed Atlas; http://hawaii.gov/dlnr/dar), tissues from specimens were obtained from 6 (Molokai) to 12 (Oahu) drainage basins on each island for each species, with the exception of *S. stimpsoni* adults (i.e., post-metamorphic individuals of reproductive size / age) on Oahu (Figure 6, Table 1). Between 1 and 6 sites were sampled in each watershed in 2009 and 2011, for a total of 107 sites in 41 watersheds. Additional abiotic and biotic data (i.e., water chemistry, assemblage structure) was obtained from other watersheds where specimens were not sampled (see section 4.0.1). Both species were collected using hand nets. Because they are difficult to collect via hand netting, we also attempted to collect immigrating postlarvae at each location using modified Breder traps, as in Benbow et al. (2004). However, trap rates were sufficiently low that we abandoned the use of Breder traps for this study. Up to 15 adults and 15 postlarvae of each species were captured per location, with the exception of *S. stimpsoni* adults on Oahu (Table 1). Only *S. stimpsoni* postlarvae were captured on Oahu. This enabled us to examine ≥100 adults and ≥100 postlarvae per species from each island- with the exception of *S. stimpsoni* on Oahu- to provide the most comprehensive assessment to date of within-island and between-island patterns of genetic variation.

Each captured specimen was kept temporarily in a holding container for subsequent processing. Stage of maturity was assessed by total length measurements; we categorized individuals of *S. stimpsoni* of total length > 4.5 centimeters (cm) and *A. stamineus* > 7.5 cm as having reached adulthood (Kinzie et al. 1984, Gingerich et al. 2005). Specimens of *S. stimpsoni* between 2.5 cm and 4.5 cm and *A. stamineus* between 4.5 cm and 7.5 cm were categorized as young-of-year juveniles. We categorized *S. stimpsoni* specimens smaller than 2.5 cm and *A. stamineus* smaller than 4.5 cm as immigrating postlarvae. Clips of approximately 10-30 mg of caudal fin were taken from each juvenile and adult specimen and preserved immediately in 95% ethanol.

Awaous

Island	Watersheds Total	Sites Total	2009 Watersheds	Sites	Ntotal	Nave	Nmin	Nmax	Nseq	Ngen
Kauai	8	19	8	19	242	30	5	53	208	197
Oahu	12	35	12	30	389	32	1	69	318	274
Molokai	6	16	6	15	221	37	2	50	193	188
Maui	8	21	7	16	227	32	3	59	217	183
Hawaii	7	16	6	11	106	18	1	16	99	99
Average	**8**	**21**	**8**	**18**	**237**	**30**	**2**	**49**	**207**	**188**
Total	**41**	**107**	**39**	**91**	**1185**				**1035**	**941**

Island	2011 Watersheds	Sites	Ntotal	Nave	Nmin	Nmax	Nseq	Ngen
Kauai	6	15	241	40	16	58	216	221
Oahu	9	22	351	39	4	57	341	344
Molokai	5	14	276	55	37	67	162	268
Maui	7	16	288	41	23	70	244	280
Hawaii	6	13	254	42	26	54	239	251
Average	**7**	**16**	**282**	**44**	**21**	**61**	**240**	**273**
Total	**33**	**80**	**1410**				**1202**	**1364**

Sicyopterus

Island	Watersheds Total	Sites Total	2009 Watersheds	Sites	Ntotal	Nave	Nmin	Nmax	Nseq	Ngen
Kauai	8	17	8	15	112	14	1	45		57
Oahu	4	7	1	3	8	8	2	3		2
Molokai	5	13	5	11	95	19	1	45		72
Maui	8	19	6	13	95	16	2	48		67
Hawaii	7	13	4	5	107	27	1	38		41
Average	**6**	**14**	**5**	**9**	**83**	**17**	**1**	**36**		**48**
Total	**32**	**69**	**24**	**47**	**417**					**239**

Island	2011 Watersheds	Sites	Ntotal	Nave	Nmin	Nmax	Nseq	Ngen
Kauai	5	7	151	30	1	76		151
Oahu	3	4	16	5	2	7		14
Molokai	5	11	258	52	1	79		236
Maui	6	10	211	35	14	77		197
Hawaii	4	10	176	44	1	61		170
Average	**5**	**8**	**162**	**33**	**4**	**60**		**154**
Total	**23**	**42**	**812**					**768**

Table 1: The number (Ntotal) of *Awaous stamineus* and *Sicyopterus stimpsoni* sampled across the Hawaiian archipelago in 2009 and 2011, with reference to: the total number of watersheds (Watersheds Total) and sites (Sites Total) sampled for each species; the number of watersheds (Watersheds) and sites (Sites) sampled per island per year; the average number (Nave), minimum number (Nmin), and maximum number (Nmax) of specimens sampled per watershed; the number of specimens sequenced per island (Nseq); and the number of specimens genotyped per island (Ngen). All *Sicyopterus* sampled on Oahu were postlarvae.

Due to the difficulty of identifying postlarvae to species by morphology (Tate et al. 1992, Lindstrom 1999), all immigrating postlarvae were sacrificed for genetic analysis. All juveniles

and adults were released after being processed except for a subset of *A. stamineus* adults that were sacrificed for the study of otolith microchemistry (see below).

***DNA extraction and mitochondrial DNA sequencing*:** Genomic DNA was recovered from fin tissue or lateral muscle samples (from specimens sacrificed for otolith analysis) using DNeasy (Qiagen) extraction kit designed for animal tissue. For both species, the complete mitochondrial Cytochrome b (cytb) gene was amplified using approximately 10 nanograms (ηg) of template DNA and two primers, GluFish (5'–ACCACCGTTGTTATTCA ACTACAA–3'; and ThrFish2, 5'–AACCTCCGACATCCGGCTTACAAGACCG–3') in a 15 milliliter (ml) polymerase chain reaction (PCR) cocktail consisting of a final concentration of 1X PCR buffer, which includes 2.0 millimolar (mM) Magnesium Chloride ($MgCl_2$), 0.5 mM $MgCl_2$, 0.16 mM of each deoxyribonucleotide (dNTP), 0.5 mM of each primer, and 0.3 units (U) Paq or HoTaq DNA polymerase (MCLAB Inc.). Thermal cycling conditions consisted of an initial denaturation step at 94^o Celsius (C) for 6 minutes (min), followed by 35 cycles of 94^{o}C for 15 seconds (s), 52^{o}C for 15 s, 72^{o}C for 15 s, and a final extension at 72^{o}C for 10 min using MBS Satellite 0.2G thermo cyclers (Thermo Electron Corporation).

PCR amplicons were purified using ExoSAP-It (USB, Affymetrix) and cycle sequencing reactions were performed using FishSeq (5'–CCACCGTTGTTATTCAACT ACAAG–3') and ThrFish2 primers and BigDye 3.1 chemistry (Applied Biosystems) to characterize a ~ 500 base pair (bp) section of the cytb gene for both species.

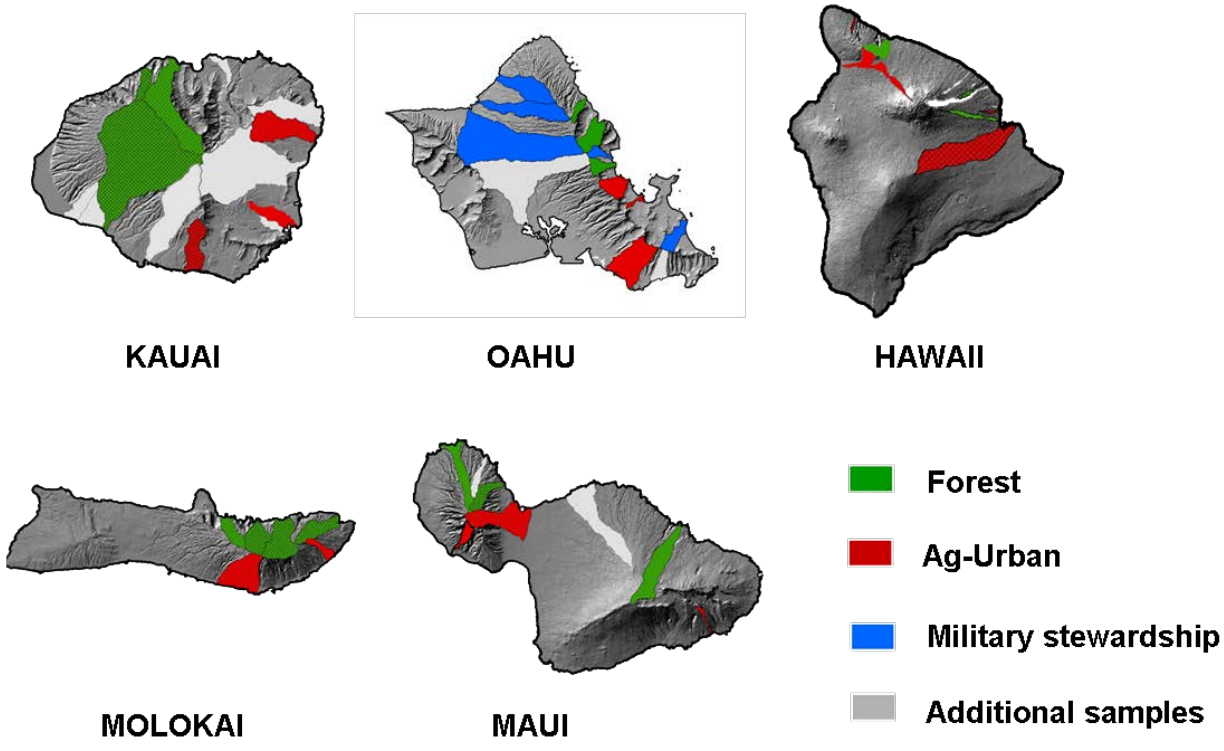

Figure 6: Watersheds where abiotic and biotic data were obtained and where *A. stamineus* and/or *S. stimpsoni* were sampled in the Hawaiian archipelago, categorized according to dominant land use or stewardship. Watersheds where additional reference samples or survey data were obtained also are identified.

Because we recovered a relatively small number of cytb haplotypes for *S. stimpsoni*, we also examined sequence data for a ~680 bp region of the mitochondrial Cytochrome Oxidase I (CO1) gene. Amplification and sequencing conditions were identical to those used for the cytb region, except primers L6468 (5'–GCTCAGCCATTTTACCTGTG–3') and H7696 (5'–AGGCCT AGGAAGTGTTGAGGGAAG–3') were included in the PCR cocktail (Thacker and Hardman 2005). Thermal cycling conditions consisted of an initial denaturation step 94^{o}C for 5 min, followed by 30 cycles of 94^{o}C for 40s, 55^{o}C for 60s, 72^{o}C for 1.5 min, and a final extension at 72^{o}C for 5 min. Cycle sequencing reactions were performed using primers SsC01INTF1 (5'–CCCCTCTAGCAGGA AACCTT–3') and SsC01IntR2 (5'–TACAAGACAGATACCCCCGT–3') and BigDye 3.1 chemistry (Applied Biosystems).

All sequencing reactions were purified using Sephadex (GE Healthcare Biosciences) plate protocols and analyzed on an ABI 3100 or ABI 3730xl automated DNA sequencer (Applied Biosystems).

Awaous microsatellite genotyping: Tissue clips were used to genotype *A. stamineus* at 13 microsatellite loci. Nine of the loci have been published in Hogan et al. (2010): Agua A4, Agua B1, Agua B2, Agua C4, Agua D3, Agua D9, Agua D103, Agua D110, and Agua D135. Of the remaining loci, Agua D106, exhibits a repeat motif of $(CTAT)_5$ and an optimal annealing temperature of 52°C. Primer sequences for amplification are (F) TCACCTGGTCACTGATT ATG and (R) GGGAGGCG AACTTTGAGAG. Locus Agua D117 exhibits a repeat motif of $(TATC)_9$ and an optimal annealing temperature of 48°C. Primer sequences for amplification are (F) GCCAAAACTCATACAAGAGGT and (R) GGTCTGCTTCTGAAATCTC. Locus Agua D6 exhibits a repeat motif of $(TCTA)_{12}$ and an optimal annealing temperature of 53°C. Primer sequences for amplification are (F) GCCCTAAACTGTGGAACTCT and (R) AGTGCGAACA AGTGTGTTTC. Locus Agua D7 exhibits a repeat motif of $(AGAT)_5$ and an optimal annealing temperature of 51°C. Primer sequences for amplification are (F) CTCCCAGCAATGTTATA GGT and (R) GGATCTATGGGAGCTGTACC.

PCRs were performed in 15 µL reaction volumes with conditions following Hogan et al. (2010). Each reaction contained 10 ηg of template DNA, 1X PCR buffer (contains 2mM $MgCl_2$), 0.33 mM of each primer, 0.33 mM of each dNTP and 0.25 U Taq polymerase (MCLAB Inc.). PCR products were amplified using MBS Satellite 0.2G thermo cyclers (Thermo Electron Corporation) with the following conditions: a 10 min hot start at 95ºC followed by 32 cycles of 94ºC for 15s, a locus specific annealing temperature (Hogan et al. 2010) for 15s and 72ºC for 30s, followed by a final elongation step at 72ºC for 6 min. HEX, 6-FAM or NED fluorescently dye-labeled forward primers were used to generate labeled PCR amplicons for sizing the loci against a 500 ROX™ or LIZ™ size standard on an ABI 3100 or ABI 3730xl DNA analyzer. Electropherograms were scored using GENEMARKER v1.9 (SoftGenetics LLC).

Sicyopterus microsatellite genotyping: Tissue clips were used to genotype *S. stimpsoni* at 12 microsatellite loci (Table 2). The microsatellite markers were developed for *S. stimpsoni* from a partial genomic library enriched for CA-repeats (Moody et al., unpublished data). The loci were initially tested on 80 adult fish from the islands of Kauai and Hawaii. The exploratory test demonstrated that the loci were highly polymorphic. The number of alleles per locus ranged

from 22 to 43, and observed and expected levels of heterozygosity per locus ranged from 0.41 to 0.97 (mean = 0.84) and 0.72 to 0.99 (mean = 0.95), respectively (Moody et al., unpublished data).

Methods for amplifying, sizing and scoring of these loci were identical to those conducted for microsatellite genotyping of *A. stamineus* as described above, with the exception of locus-specific annealing temperatures (Table 2).

Mitochondrial DNA analysis: All raw sequence files were edited, assembled, and aligned with Sequencher v4.9 (Gene Codes Corp., Ann Arbor, MI). One homologous cytb sequence of the rhyacicthyid gobioid *Rhyacicthys aspro* (specimen NSNT-P 67357, GenBank accession number AP004454) was obtained (Miya et al. 2003) to include as an outgroup taxon in phylogenetic analyses, as it is recognized as a basal taxon for all gobioids (Pezold 1993, Keith et al. 2011).

Locus	Primer Sequences (5'-3')	Alleles	Range (bp)	T_a (°C)	H_E	H_O
	F: ACTCGATTCAGTCCAATGCT					
SsA06	R_{VIC}: TCCAAACCCCGAGTCAATA	35	180-546	59	0.95815	0.412905
	F: CTCCGTCTCCATCCACAACT					
SsB07	R_{6FAM}: AACTCTCCACTGTAGCCGC	22	141-217	58	0.92941	0.8114
	F: GCGGACATACAGGTATCGG					
SsD11	R_{NED}: CATAGACCTCGATCAGGGA	31	146-252	59	0.962705	0.86153
	F: ACGCAGGCTTTAATCCGTAA					
SsC06	R_{PET}: GCCTGACGACAACAAGAAC	35	241-365	59	0.7237	0.87218
	F: GAGGAGATCCAGGACCAAG					
SsB10b	R_{VIC}: AGCTTGCAGTTTTCCCACAC	46	220-424	58	0.979035	0.973685
	F: CACCAACACACTCTGGACCT					
SsB10	R_{6FAM}: GGGTAGAGTCTGGTGTCCC	40	150-316	60	0.97897	0.89972
	F:TTCTGCAGCTTTCACCACTG					
SsC03	R_{NED}: CCATGCAGCTCCTGTGATTA	44	196-336	57	0.984425	0.936715
	F: GACCTGGTTCCTCAGTTTCG					
SsE06	R_{VIC}: GCACAGTGATGTTCTCCCT	43	194-398	59	0.980565	0.964285
	F: TTCCCCCTTGTTAGCATTTG					
SsH05	R_{6FAM}: TAATTTGCCGGAACACATCA	40	214-318	60	0.9739	0.964
	F: CCCACAAGAGATGTTCCCAT					
SsG01	R_{NED}: AAGTGCACACACGGTCACA	44	152-244	61	0.98084	0.783835
	F: GTTTGAGAAAGTCCCGCTTG					
SsG07	R_{VIC}: AAGTCAACACGATGAACCCC	30	205-301	59	0.96351	0.850875
	F: CTGAAACACTGACATGCGCT					
SsC08	R_{PET}: GCTGCATCTTCTCCCACACT	43	272-441	59	0.98814	0.80326

Table 2: Microsatellite loci developed for *Sicyopterus stimpsoni* in the Hawaiian Islands with information provided on: the number of alleles (Alleles); annealing temperature (T_a); and expected (H_E) and observed (H_O) heterozygosity averaged between Kauai and Hawaii (K. Moody, unpublished data).

Nucleotide composition of the sequenced cytb and CO1 regions was examined for variable sites with Sequencher v4.9 and GENALEX v6.5 (Peakall and Smouse 2012). Identification of unique haplotypes, haplotype frequencies at each sample location, and construction of a statistical-parsimony haplotype network using either the cytb or CO1 data also were performed in TCS 1.21 with the probability of the network set at 95% (Clement et al. 2000).

Geographic patterns of genetic variation were initially inferred from the distribution and frequency of cytb and CO1 haplotypes within and among sample sites. Patterns of genetic variation were assessed according to the frequency of haplotype classes, where haplotypes found at multiple locations were distinguished from haplotypes that only occurred at a single location. Haplotypes were also categorized according to number of intervening mutation steps in the haplotype network. Estimates of haplotype diversity, pairwise base pair substitutions among haplotypes, and nucleotide diversity were estimated using ARLEQUIN v3.1 (Excoffier et al. 2005). Pairwise genetic differentiation (Φ_{ST}), with equal weighting attributed to transitions and transversions, among sample sites was calculated using ARLEQUIN v3.1. The statistical significance of pairwise Φ_{ST} values was determined using graphically-sharpened false discovery rate (Benjamini and Hochberg 2000). To evaluate the proportion of genetic variance explained by alternative biogeographic hypotheses (Figure 2), hierarchical Analyses of Molecular Variance (AMOVA) were performed with 10,000 permutations in ARLEQUIN v3.1. Trends in relationships between pair-wise geographic distance and Φ_{ST} genetic distance values among sample sites were also examined. Finally, historical demography was analyzed using mismatch distribution tests available in ARLEQUIN v3.1, where the number of pairwise differences among haplotypes serves to determine whether the recent history of the populations deviates from the expectations under a model of sudden expansion.

Microsatellite analysis: For both *A. stamineus* and *S. stimpsoni*, a suite of genetic diversity indices were estimated for each site and for each watershed. All analyses were run with and without immigrant postlarvae. Expected heterozygosity, Shannon diversity index values, and the number of alleles were calculated using MICROSATELLITE ANALYSER (MSA) v4.05 (Dieringer and Schlotterer 2002). We also used MSA v4.05 to calculate allelic richness where values were rarified to reflect the smallest sample size among the set of sites or watersheds being analyzed.

We estimated patterns of genetic differentiation among sites within each watershed, among watersheds, and among islands for each species using several methods. Analyses were run with and without immigrant postlarvae, as warranted. First, ARLEQUIN v3.1 was used to analyze the hierarchical structure of molecular variance with sites nested within watersheds and with watersheds nested within islands (Michalakis and Excoffier 1996). MSA v4.05 was used to assess population subdivision by calculating pairwise F_{ST} values, with values bootstrapped over 999 replications (Slatkin 1995).

Additionally, we examined population structure among watersheds and islands using STRUCTURE v2.1 (Pritchard et al. 2000), which applies a Bayesian clustering algorithm to assign individuals to groups by minimizing deviations from linkage disequilibrium. After a burn-in period of 30,000 Markov-Chain Monte Carlo (MCMC) iterations, we collected data from an additional 5×10^5 iterations for 3 replicate sets of runs with the number of populations (k)

iteratively set from 1 to 5 (i.e., to test the hypothesis of cross-island and island-specific evolutionary lineages). Separate analyses were run with data sets including and excluding immigrating postlarvae. We parameterized all runs under conditions of admixture and with allele frequencies considered independent between populations. We identified the number of populations for each data set (i.e., by species, by life history stage) based upon the greatest P(X|K) value (Pritchard et al. 2000) and the break in the slope of the distribution of P(X|K) values, which is a good predictor of the uppermost hierarchical level of genetic structure among sampled individuals (Evanno et al. 2005).

Assignment tests were used to estimate contemporary dispersal by identifying recent migrants in resident populations. First, BAYESASS v1.3 (Wilson and Rannala 2003) was used to determine the number of first generation migrants (m_1) in resident populations and the directionality of exchange among watersheds and islands, as well as genetic clusters estimated by STRUCTURE v2.1. Individuals with genotypes assigned to a different watershed / island / cluster than the location of capture were identified as first-generation migrants from another site. Estimates of m_1 were obtained from the averages of five replicate runs of 5×10^7 iterations following 3×10^7 burn-in iterations. Analyses were first restricted to juveniles and then conducted with all adults and juveniles combined in a single data set. Individuals were categorized as either adults or juveniles according to body length (see above).

We also conducted assignment tests to determine whether immigrating postlarvae originated from the watershed and/or island of capture. Following Hogan et al. (2012), we applied a rank-based assignment method involving the Monte Carlo assignment procedure implemented in GENECLASS v2.0 (Rannala and Mountain 1997) and comparisons of negative log-likelihood values. Assignment to a putative source population (ie., watershed and/or island) was contingent on two criteria being met: (1) an individual must be assigned to at least one source population with a probability of 85% in the Monte Carlo based assignment procedure; individuals with a <5% probability of belonging to another site were excluded from belonging to that source population, and individuals with a probability of between 5% and the threshold value were considered unassigned; and (2) for individuals that met the threshold criteria, we discriminated among putative source populations if the likelihood of assignment to the highest ranked source population was 50% greater than the likelihood of assignment to the next highest ranked population (Hogan et al. 2012). This enabled us to classify postlarvae as successfully assigned, successfully excluded from all locations, or un-assignable with any confidence.

4.0.2 Otolith Microchemistry Analysis of Contemporary Connectivity

Otolith preparation and structural analysis: We examined otoliths from *Awaous stamineus*, arguably the most abundant and widespread of the amphidromous goby species endemic to the Hawaiian Islands. Individuals were collected from 35 watersheds (Figure 7, Table 3) across the five islands with perennial streams (total n = 383 fish; stream n= 1–23 fish; Table 3). The sampled streams were representative of the diversity of habitats available to *A. stamineus* across the archipelago and constitute ~25% of the 133 streams known to harbor *A. stamineus* (http://hawaii. gov/dlnr/dar). Sampling was conducted in 2009. Fish were collected by snorkelers

with hand nets, measured for total length, euthanized, and transported on ice to a laboratory facility where they were stored at -20^{o}C.

Island	Watershed	Estuary	N	SW Total	SW1	SW2	SW3	Unassigned	FW Total
Hawaii			**55**	**0.39**	**0.18**	**0.11**	**0.05**	**0.05**	**0.61**
Leeward	Niulii	N	4	0	0	0	0	0	1
Windward	Honolii	N	15	0.43	0.21	0	0.07	0.14	0.57
Windward	Kaieie	N	7	0.71	0.43	0.29	0	0	0.29
Windward	Nanue	N	2	0	0	0	0	0	1
Windward	Wailoa R.	Y	15	0.13	0	0.13	0	0	0.88
Windward	Waipio	Y	12	0.5	0.2	0.2	0.1	0	0.5
Kauai			**91**	**0.44**	**0.1**	**0.07**	**0.24**	**0.04**	**0.56**
Leeward	Hanakapiai	N	9	0.2	0	0.2	0	0	0.8
Leeward	Hanalei	Y	5	0.8	0	0	0.6	0.2	0.2
Leeward	Lawai	Y	20	0.47	0.06	0.06	0.29	0.06	0.53
Leeward	Waimea R.	Y	17	0.38	0.23	0	0.15	0	0.62
Leeward	Wainiha	Y	8	0.38	0.13	0	0.13	0.13	0.63
Windward	Anahola	Y	6	0.33	0	0	0.33	0	0.67
Windward	Kapaa	Y	11	0.4	0.1	0.1	0.2	0	0.6
Windward	Moloaa	N	15	0.55	0.09	0.18	0.27	0	0.45
Maui			**50**	**0.34**	**0.14**	**0.09**	**0.09**	**0.02**	**0.66**
Leeward	Alelele	N	9	0.22	0.11	0	0.11	0	0.78
Leeward	Honokohau	N	7	0.29	0	0	0.14	0.14	0.71
Windward	Iao	N	7	0.33	0.33	0	0	0	0.67
Windward	Kahakuloa	N	2	0	0	0	0	0	1
Windward	Piinaau	Y	13	0.56	0.11	0.44	0	0	0.44
Windward	Waihee R.	N	12	0.36	0.18	0	0.18	0	0.64
Molokai			**78**	**0.33**	**0.05**	**0.22**	**0.05**	**0.02**	**0.67**
Leeward	Honouli Wa	N	19	0.41	0.06	0.29	0.06	0	0.59
Leeward	Kamalo	Y	1	0	0	0	0	0	1
Windward	Halawa	Y	23	0.33	0.06	0.17	0.06	0.06	0.67
Windward	Pelekunu	N	20	0.29	0.06	0.18	0.06	0	0.71
Windward	Wailau	N	15	0.3	0	0.3	0	0	0.7
Oahu			**109**	**0.39**	**0.24**	**0.06**	**0.05**	**0.03**	**0.61**
Leeward	Ala Wai	Y	19	0.37	0.26	0	0.11	0	0.63
Leeward	Kiikii	Y	1	0	0	0	0	0	0
Leeward	Waimea	Y	4	1	1	0	0	0	0
Windward	Kahaluu	Y	6	0.14	0.14	0	0	0	0.86
Windward	Kahana	Y	12	0.54	0.15	0.15	0.08	0.15	0.46
Windward	Kaluanui	N	1	1	0	0	0	1	0
Windward	Keaahala	N	22	0.28	0.22	0.06	0	0	0.72
Windward	Waiahole	N	14	0.33	0.25	0	0.08	0	0.67
Windward	Waikane	N	16	0.5	0.36	0.14	0	0	0.5
Windward	Waimanalo	Y	14	0.3	0.1	0.1	0.1	0	0.7
Totals			**383**	**0.38**	**0.14**	**0.11**	**0.09**	**0.03**	**0.62**

Table 3: The number of fish (N) sampled per island and watershed included in the study of otolith microchemistry. Watersheds are identified as being on either the windward or leeward sides of the islands as well as whether they have an estuary or not. The proportion of fishes collected on each island and in each watershed are identified according to whether individuals exhibited marine (SW, SW1, SW2, SW3), unassigned, and freshwater (FW) larval chemistry.

In the lab, fish were thawed and sagittal otoliths were extracted following standard protocols (Bickford and Hannigan 2005). For each fish, sagittal otoliths were removed and cleaned in distilled water and allowed to dry. All tools used for extraction were Teflon coated and acid washed to reduce contamination. One otolith was randomly selected from the pair and mounted for microchemical analysis. The same otolith was also analysed for microstructure, including age and growth measurements. Selected otoliths were mounted sulcus side up onto petrographic slides and embedded in Crystal Bond glue. The mounted otoliths were ground and polished in the sagittal plane, using fine grit and velvet polishing pads (Buehler), to expose the daily growth rings from the edges to the primordium.

We measured life history characteristics from the daily-ring structure of otoliths from 216 fish representing 33 of the 35 study watersheds. These characteristics included hatching and metamorphosis dates, age, larval duration, otolith size at metamorphosis, larval growth rates, and adult growth rates. The life history was divided into larval and post-metamorphic phases based on the presence of a metamorphic mark, a relatively broad ring having high optical density that arises during metamorphosis from the larval form into the postlarval form (Radtke et al. 1988). Age at capture and larval duration were estimated by counting otolith rings, referred to as "radii", which accrue daily (Radtke et al. 1988). Radii were measured from primordium to the metamorphic mark and from the metamorphic mark to the edge of the otolith. The radii are used as a course measurement of larval size and growth and post-settlement size and growth (Campana and Jones 1992, Sponaugle et al. 2006). Otoliths were examined by three independent readers, and the mean age estimate was used when two or more readers agreed (coefficient of variation, CV <10%). If there was not agreement or otolith condition was too poor for reader confidence, age was not estimated.

Hatching dates and metamorphosis dates were back-calculated from the date of collection. Larval size at metamorphosis was measured as the otolith radius from the first ring to the metamorphic mark. Larval and post-metamorphic growth rates were calculated from the otoliths based on the expectation that otolith growth is closely correlated with somatic growth (Campana and Jones 1992). We used an exponential model to estimate growth rate (following Shima and Swearer 2009):

$$L_t = L_0 (e^{K(t)})$$

where L_t is the otolith radius at time t, L_0 is the otolith radius at time zero, and K is the instantaneous growth rate parameter (Shima and Swearer 2009). L_t for larval growth was the radius from the primordium to the metamorphic mark, and L_t for post-metamorphic growth was the radius from metamorphic mark to otolith edge. For larval growth, L_0 was set at 0.01; for post-metamorphic growth, L_0 was the radius from primordium to the metamorphic mark.

Otolith microchemical analysis: Otoliths from all 383 individuals were analyzed with LA-ICP-MS (Cetac LSX213 and Perkin Elmer ELAN DRCII) at the University of Massachusetts, Boston. Samples were analyzed with a laser transect starting at one edge of the otolith, bisecting the primordium and ending at the other edge of the otolith, resulting in a palindromic signal for all samples. For all samples and standards, LA-ICP-MS involved use of a laser beam width of 25 micrometers (µm) and a scan rate of 15 $\mu m \cdot s^{-1}$. Eighteen isotopes were analyzed, and concentrations were calculated for the larval and post-metamorphic regions of each otolith

transect using GeoPro software (Cetac). Relative concentrations were expressed as a ratio relative to ^{46}Ca. Six isotopes were considered informative for statistical analysis based on signal to noise ratio considerations, including: ^{55}Mn, ^{85}Rubium (Rb), ^{88}Sr, ^{138}Ba, ^{66}Zinc (Zn) and ^{63}Copper (Cu). Finally, 15% of the total sample set was excluded from statistical analysis because more than half of their isotope ratios were outliers (>3 Standard deviations (SD) from overall mean).

All samples were calibrated and drift corrected using two calcium carbonate standards (US Geological Survey (USGS, MACS-1, MACS-3) and background corrected against the argon carrier gas from a gas blank taken before each sample analysis. ^{43}Ca was used as an internal standard to compensate for signal variation caused by differences in mass of ablated material.

To confirm the veracity of the finding from LA-ICP-MS that some fishes did not go to sea as larvae, wavelength dispersive X-ray spectroscopy was conducted using an electron microprobe at the University of Wisconsin, Madison. Laser beam depth using LA-ICP-MS was up to 30 µm deep, which could introduce *z*-dimension bias and wash out a true high strontium signal (i.e., ocean-going) if strontium cores were superficial. Electron probe analysis samples the first 1-3 µm of the otolith surface, therefore eliminating the possibility of *z*-dimension bias. Eight independent pilot samples were used to confirm the findings of LA-ICP-MS (three samples with high Sr cores and five with low Sr cores). Electron probe samples were taken using a 20 µm defocused beam. Twenty to thirty spot samples were taken parallel with the original LA-ICP-MS transect from edge to core. Strontium and calcium were measured as weight percent (wt%) and calibrated with a UWC-3 calcite standard.

Otolith-based analysis of dispersal histories: Based on the analyte list, counts (cps) for all 18 isotopes were acquired approximately every one second that the laser was firing. Background gas signals were integrated over an approximately 25 s window (Figure 8), MACS standard integrations were also approximately every 25 s. Edge signals were integrated over a 15 s window, and core signals were on average 15 s long (Figure 8). Visual analysis revealed that the average length of a strontium peak in the otolith core, from primordium outward, was 15 s. Furthermore, the primordium was, on average, centered on the palindrome transect. Not all otoliths showed visual evidence of a strontium peak in the core. For these samples, we selected the core region starting in the middle of the signal and going outward 15 s from the midpoint.

After signal integration and parts per million (ppm) concentrations were calculated, all isotopes were expressed as a ratio relative to ^{46}Ca. A shortened isotope list was selected for statistical analysis. If multiple isotopes of the same element were measured, a strong correlation between the isotopes was confirmed, and then the most abundant isotope was used for analyses. Isotopes were further selected via a two-step process by comparing the “left” and “right” sides of the palindrome transect. First, the mean intra-individual CV was calculated for each isotope by taking the mean and standard deviation of the left and right side of the signal for each sample. An isotope was excluded from statistical analyses if the mean intra-individual CV was greater than one, indicating a high level of variation between the left and right side of the otolith transect. Second, an isotope was excluded from statistical analyses if it was poorly correlated ($r^2 < 0.3$) between the left and right sides of the palindrome.

Otolith chemistry was used to address two sequential questions about larval dispersal history: (1) is amphidromy obligate in *A. stamineus*, or do some larvae remain in freshwater throughout development? and (2) do larvae that undergo marine dispersal follow different dispersal pathways in the ocean? The Sr:Ca ratio in the larval phase was used to determine whether larvae exhibited amphidromy. Saltwater residency is indicated by elevated Sr:Ca ratios, and has been widely used to identify marine habitat use in diadromous fishes (e.g., Michel et al. 2008). Strontium peaks in the larval phase were easily identifiable and Sr:Ca declined rapidly after the metamorphic mark (Figure 8). Conversely, otoliths with no evidence of marine dispersal were identified by having a low and constant Sr:Ca ratio throughout the larval and post-metamorphic phases.

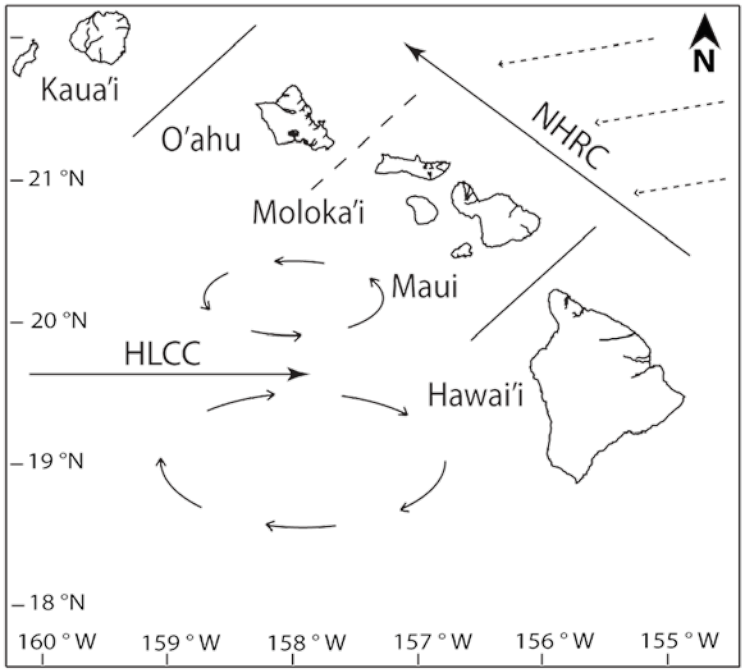

Figure 7: Map of the Hawaiian archipelago, with the streams (black lines) sampled for the study of otolith microchemistry. Solid arrows indicate major surface currents within the archipelago; the Hawaiian Lee Countercurrent and the North Hawaiian Ridge Current including two circulations. Dashed arrows indicate the direction of prevailing winds. Lines separating the islands indicate the position of consensus (solid line) and putative (dashed line) genetic breaks for marine species within the archipelago (redrawn from Toonen et al. 2011).

We used principle components analysis (PCA) of otolith microchemistry during the post-metamorphic and larval phases to test whether putatively freshwater (FW) larvae were similar in chemistry to the post-metamorphic period which is unquestionably spent in freshwater. PCA was conducted on a restricted set of isotopes. To confirm the inference from LA-ICP-MS that some larvae never went to sea, otoliths from eight independent samples were also analyzed with wavelength dispersive X-ray spectroscopy using an electron microprobe (EPMA). The EPMA transect paralleled the LA-ICP-MS transect on the same otolith, and yielded data on the Sr:Ca ratio only.

We first tested whether average microchemical signatures during marine dispersal were distinct at the island or watershed scale. Following multivariate analysis of variance (MANOVA) comparisons, we used classification success from discriminant function analysis (DFA) to quantify the strength of spatial structuring by island and watershed. A Mantel test (*vegan* package in R v2.13.1) was used to determine whether otolith microchemistry during the larval phase differed by geographical distance at the scale of islands. MANOVA and DFA analyses were performed in Statistica v.10 (StatSoft).

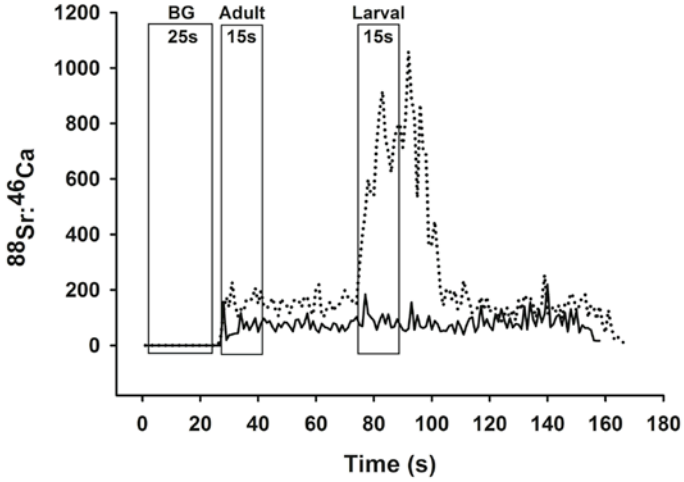

Figure 8: Example of amphidromous (dashed line) and non-amphidromous (solid line) otolith profiles; high Sr:Ca ratios in the middle of the profile indicate larval residence in saltwater. Boxes indicate the method of signal selection for each sample; a 25 s window selected for background (BG) signal, and 15 s windows for both the post-metamorphic (Adult) and Larval signals.

Additionally, we used a naïve Bayesian approach to assess the existence of natural groupings based on the otolith microchemistry corresponding to the larval period (R v2.13.1 using the *cluster.optimal* function in the *bayesclust* package). This method searches for *k* optimal clusters in the data with no prior information about group structure. Runs of 10 million simulations were performed for $k = 2$ through $k = 6$. The best five simulations from each level of *k* were kept to test whether the replicate simulations produced the same assignment for each individual. Those individuals that were assigned to the same cluster in all 5 replicate simulations were considered assigned to that cluster. All other individuals were considered unassigned. Negative log-likelihoods were used to test which value of *k* was most likely. Five likelihoods for each *k* were output from the five best replicate simulations, and the precision of these replicate likelihoods indicates the convergence of the algorithm on this solution. Likelihoods will always increase with increasing number of groupings (*k*'s), so we used an information criterion "Delta K" (modified from Evanno et al. 2005) to identify the most parsimonious number of *k* groups. This information criterion uses the rate of change in likelihood between successive *k* groups and penalizes for within *k* variation in likelihood (i.e., poor convergence). The "true" *k* is that which exhibits the largest increase in likelihood from its predecessor, while maintaining strong convergence. The *cluster.test* function also was employed in *bayesclust* to test the hypothesis that there were no clusters in the data (i.e., $k = 1$). This function calculates an empirical probability that the true value of *k* is >1 by comparing simulations assuming $k > 1$ to a null distribution. Simulations were performed for $k = 2$ through $k = 6$ using the default setting of 500,000 simulations. The null distribution was generated using the default setting of 100 simulations. A *p*-value was calculated for each empirical probability using the *emp2pval* function in the same package.

Frequentist statistics were used to compare the naïve groupings to *a priori* groupings (i.e. islands or watersheds) based on how well they explain variation in chemistry. MANOVA was used to test for differences between the *k* groups, PCA was used to visualize differences in chemistry between the *k* groups, and DFA was used to assess classification strength of the naïve Bayesian groupings.

There are several hypotheses that could explain chemical differences during the marine dispersal phase, including temporal variation in ocean chemistry, larval residence in estuaries or bays, leeward vs. windward differences in ocean chemistry, and near-shore vs. off-shore larval residency. To test for temporal effects, we used z-tests to compare hatching and settlement dates among the *k* groups. Similarly, to test for geographical effects, the proportions of larvae from the *k* groups were compared between windward and leeward watersheds, or watersheds with and without estuaries. Concentrations of Cu (elevated by anthropogenic inputs along coasts; Forrester and Swearer 2002) were compared among the *k* groups to test whether marine clusters were related to on-shore vs. off-shore larval residency. The correlation between post-metamorphic (stream) and larval (marine) Cu:Ca was used to evaluate whether larvae resided near or in a freshwater environment, such as the estuary of the stream that they recruited to as postlarvae (i.e., where they were collected).

After categorizing the dispersal history of each fish (freshwater-only or one of *k* marine groups), we used larval duration and growth rates to assess the fitness implications of each dispersal group. We compared the frequency distributions of larval hatch dates, larval settlement dates, larval durations (LD), size-at-settlement, larval growth, and adult growth rates among dispersal groups using Kolmogorov-Smirnov tests. First, these quantitative life history traits were compared between amphidromous and non-amphidromous individuals (i.e., marine vs. freshwater larval phase). Then, the same comparisons were made among the *k* groups representing alternative marine dispersal pathways.

4.0.3 Use of Oxygen Isotopes in Otoliths for Reconstructing Life History

Study species and field sampling: This study focused on *Awaous stamineus*, which inhabit the lower and middle reaches of perennial streams across the Hawaiian Islands. The species lives on average 2.4 years (Hogan et al. in review), most or all of which is spent in freshwater, including maturation and spawning in the stream environment (Radtke et al. 1988, Hogan et al. in review). Peak spawning is from August through December (Ha and Kinzie 1996). After hatching, larvae migrate downstream towards the ocean (McDowall 2007). Though some larvae spend as much as 5 months at sea (Radtke et al. 1988), not all enter the marine environment (Hogan et al. in review). It is during the marine phase that larvae may disperse among watersheds and islands (Hogan et al. in review).

We collected *Awaous stamineus* postlarvae from sites at the mouths of the following five watersheds: Waimea, Kaluanui and Waimanalo watersheds on Oahu; Lawai watershed on Kauai; and Halawa watershed on Molokai. The sampled watersheds are representative of land use gradients (i.e., forested to urban) and stream water temperatures (21.1 – 24.5°C) across the archipelago. Sampling was conducted from April to June 2011. Fish were collected by snorkelers

with hand nets, measured for total length, euthanized, and transported on ice to a laboratory facility where they were stored at -20^{o}C.

Otolith preparation and microstructure analyses: Detailed age and daily growth data was collected for nine *Awaous* individuals. After thawing each specimen, sagittal otoliths were extracted using Teflon coated forceps and a fine-bristled paint brush. The otoliths were then cleared of soft tissue, dried, and stored in 1.5 ml polypropylene vials. For each fish, one otolith was randomly selected for microstructure analysis of daily growth rings and LA-ICP-MS trace element analysis. The selected otolith was mounted on to a petrographic slide in Crystal Bond glue and polished in the sagittal plane using fine-grit diamond coated lapping films (3M) to reveal the daily growth rings from the primordium to the edge. Photographic images were taken with a Leica DFC 295 digital camera mounted to a compound microscope using a 20X objective lens. A composite image was compiled in Adobe Photoshop, from which daily growth rings were counted and increment widths measured using Leica Application Suite software.

Age and daily growth before and after metamorphosis were determined based upon a clearly-visible metamorphic mark on the otolith characterized by a thick band of high optical density (Radtke et al. 1988). Larval age was measured as to the number of daily rings from the primordium to the metamorphosis mark. Postlarval age corresponded to the number of daily rings from the metamorphosis mark to the otolith edge. Daily growth was measured across the otolith radius for both larval and postlarval growth periods, with growth measured as the width of each daily increment.

Preliminary comparisons showed that all *Awaous* individuals exhibit particularly fast growth rates in the early larval growth period, compared with later growth rates as pre-metamorphic larvae. We therefore determined variation among individuals in the duration of early larval growth by subdividing the larval growth periods into early (ELG) and late larval growth (LLG) periods. The duration of the ELG period was determined for each individual by subtracting the mean larval growth rate (MLG) from each daily larval increment. Anomalous growth was determined as any daily increment that was larger than the MLG rate. The duration of the early larval growth anomaly was determined for each individual by counting the number of daily rings that were above the MLG rate. This approach showed that the anomalous ELG period was complete when daily growth rings were above the average growth rate for less than 3 consecutive days. The LLG period thus corresponded to the remainder of the larval period (prior to the metamorphosis mark), in which larval growth was slower than the MLG daily growth rate.

LA-ICP-MS analysis: The same otolith used for microstructural analysis was subsequently analyzed for trace element chemistry by LA-ICP-MS. Otolith samples were triple-rinsed with Milli-Q water and dried prior to analysis. All otoliths were analysed with LA-ICP-MS (Cetac LSX213, Perkin Elmer ELAN DRCII) at the Analytical Geochemistry Laboratory at the University of Massachusetts, Boston. Samples were analyzed with a laser transect (beam width: 25µm; scan rate: 15µm$\cdot$s^{-1}) starting at one edge of the otolith, bisecting the primordium and ending at the other edge of the otolith, resulting in a palindromic signal.

Nineteen isotopes were analyzed sequentially, yielding one measurement of each isotope every 1.16 s. All samples were calibrated and drift corrected using two calcium carbonate standards

(USGS MACS-1, MACS-3). Background argon carrier gas concentrations of each isotope were subtracted from each otolith sample. Concentrations (ppm) were calculated for each measurement across the transect using GeoPro software (Cetac). The ten elements that were consistently detectable above background gas levels (Sr, Ba, Mg, Mn, Chromium (Cr), Nickel (Ni), Cu, Zn, Lanthanum (La), Pb) were used in statistical analyses. Relative concentrations of all isotopes were expressed as a ratio to ^{46}Ca.

Concentrations of the ten elements were measured for all nine *Awaous* individuals to determine changes in habitat use across life history stages. Sr:Ca was used as an indication of changes in salinity (Zimmerman 2005), with the expectation that *A. stamineus* larvae transition to freshwater at or around metamorphosis (Radtke et al. 1988). By comparing Sr:Ca to the location of the metamorphosis mark, we examined whether Sr:Ca serves as an accurate indicator of transitions to freshwater. We used all ten elements to determine whether larval growth anomalies (ELG) were related to particular chemical environments (i.e., habitats) compared to basal growth periods (LLG). We predicted that individual variation in anomaly duration would be linked to different chemical environments reflecting patterns of habitat use. We used student t-tests and regression analysis to test for associations between larval growth and habitat use.

Secondary ion mass spectrometry analysis: The second otoliths extracted from each of the nine individuals were roasted in a vacuum oven at 262 °C for 2 hours to remove protein and other organic materials. For roasting, otoliths were mounted in Buehler EpoxiCure epoxy resin on a glass coverslip with the sulcus facing upward (i.e., away from glass). A UWC-3 calcite standard was mounted adjacent to the otolith. Once hardened, the mounted otolith and standard were embedded facing downward in a 25.4 mm epoxy round (i.e., the glass coverslip was located on the surface of the round. SIMS analysis demands precisely flat and smooth samples with a vertical tolerance of <10 µm. To achieve this tolerance, the epoxy rounds were first ground with a 6 µm diamond mesh on a Buehler polishing wheel until the glass covering the otolith and standard was ground away. Samples were then polished with fine-grit diamond slurries (range: 3 µm – 0.05 µm) to ensure that the entire growth surface was exposed from primordium to edge and to ensure flat and smooth sample surfaces. Epoxy rounds with samples and standards were triple rinsed with distilled water and ethanol, dried in a nitrogen (N_2) atmosphere, and gold-coated for SIMS analysis.

Oxygen isotopes and hydroxide (^{16}OH) anions were measured with the CAMECA-IMS-1280 ion microprobe at the University of Wisconsin, Madison. The primary ion beam was $^{133}Cs^+$ set at 2.5 nA for 3.5 min for $\delta^{18}O$ spot samples. Sample spots were ovoid with a length of 10 µm and width of 5 µm. SIMS spots were analyzed across the otolith surface in a transect from primordium to edge along the longest axis. Ions were extracted with 10 kilovolts (kV) and selected using a 40 electronvolt (eV) energy window. Multiple Faraday cups were used to simultaneously measure ^{18}O, ^{16}O and ^{16}OH. Calcite standards were measured every ~8 sample spots to drift correct otolith samples. Oxygen isotope ratios are expressed using per mil (‰) notation relative to Standard Mean Ocean Water.

Though concentrations of $\delta^{18}O$ were measured for all nine *Awaous* individuals, $\delta^{18}O$ profiles for four individuals were not used in subsequent analyses. The ion beam of the ion microprobe penetrates approximately 3 µm deep into the sample surface, and so samples must be prepared

with extreme precision in order to expose the otolith growth surface from primordium to edge. A preliminary assessment of $\delta^{18}O$ patterns in the four excluded fish suggested that their respective otoliths did not fall within the tolerance window, hence the data were not reflective of the individuals' entire life cycle.

Because $\delta^{18}O$ is expected to be depleted in freshwater, we assessed whether $\delta^{18}O$ serves as an indicator of larvae transitioning from marine to freshwater habitats. In addition, we assessed whether shifts in $\delta^{18}O$ align with metamorphosis, and we compared the relative precision of $\delta^{18}O$ with Sr:Ca as an indicator of marine to freshwater habitat transitions. We also investigated variation in $\delta^{18}O$ within the larval and postlarval periods. Assuming a constant end-member $\delta^{18}O$ within marine and freshwater environments, changes in $\delta^{18}O$ within either environment could be interpreted as within-environment changes in temperature or salinity.

4.0.4 Coupled Biophysical Modeling of Larval Dispersal

Model descriptions and parameters: We employed the high resolution 3D oceanographic model developed for the Hawaii region by the Hybrid Coordinate Ocean Model (HYCOM) consortium (see http://hycom.org/hycom/overview for a detailed description of the HYCOM program). The HYCOM circulation model covers the full Hawaiian archipelago (16° N – 26° N and 166° W to 150° W) at a resolution of $1/25^{th}$ or a degree latitude and longitude (~4 km resolution). Daily outputs from this model (which are freely available on the web via portals such as the Asia-Pacific Data Research Center; http://apdrc.soest.hawaii/) include estimates of sea surface temperature, salinity, as well as eastward, northward and upward current flows.

Outputs from the HYCOM model were used to inform a particle tracking algorithm (Figure 9) developed to estimate connectivity of marine fish and invertebrate populations across the Hawaiian archipelago and the central Pacific (Polovina et al. 1999, Kobayashi 2006, Kobayashi and Polovina 2006). We used a version of the Lagrangian transport model described in Polovina et al. (1999) with the following modifications: (1) the current product used was upgraded from 8-day Topex/POSEIDON to the daily regional HYCOM current product (Jia et al. 2011) from 2 May 2009 until 1 November 2011; (2) the diffusivity coefficient was set to 250 m^2 / s ; and (3) a 5 km settlement radius was implemented. These modifications were similar to those used by Rivera et al. (2011), but include a further refinement of the diffusivity coefficient (Y. Jia, personal communication).

The particle tracking algorithm was further modified to examine outcomes of variation in larval life duration (Radtke et al. 1988, Bell and Brown 1995, Kingsford et al. 2002) and variation in larval production (e.g., the number of larvae that enter the oceanographic model; see Kobayashi 2006 for an example).In an effort to best represent the apparent bimodal marine dispersal potential of *A stamineus* (Radtke et al. 1988, Hogan et al. in review), we examined outcomes of two different LLDs: 55 days and 150 days. Virtual larvae were released daily from stream mouth locations for 857 days and 763 days for the 55 and 150 day LLD runs, respectively, in an effort to temporally maximize utilization of the available HYCOM current data. A total of 51 stream mouth locations on the islands on Hawaii, Maui, Molokai, Oahu and Kauai were used as both release and settlement sites (Figure 10). For these sets of simulations, all larvae were

assumed to be passive throughout their LLD, in all model runs. We ran a model simulation for two different spawning scenarios: one constant spawning throughout the year, and the other scaling spawning to relative stream flow rates in each watershed. The constant spawning scenario allowed us to investigate a physically driven dispersal scenario, whereas scaling spawning output to stream flow allowed us to examine more biologically realistic dispersal scenarios.

Model runs: Each run of the model involved release of a user-defined number of virtual larvae from user-defined release sites, with daily time-step tracking of dispersal according to 3D ocean currents and biological parameters of the PT model. Each run continued for a defined number of days reflecting LLD, after which the position of the larvae was recorded. If a larva ended its run within a defined radius of a stream it was considered to have successfully entered the stream environment, whereas those that failed to enter a stream radius were considered to have died during larval transport (Kobayashi 2006). Settlement radii were set at 5 km, as this is the distance over which larvae are likely to be able to detect suitable settlement habitat and actively swim towards the stream environment (Kingsford et al. 2002, Fisher and Wilson 2004).

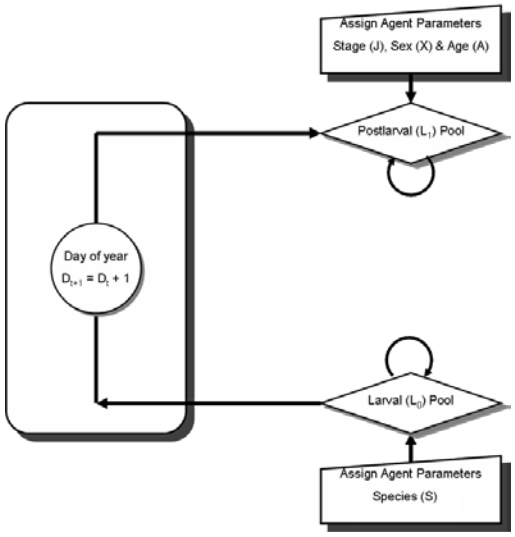

Figure 9: Structure of the coupled biophysical model implemented to estimate larval dispersal across the Hawaiian Islands. The model is composed of a particle tracking module that regulates the number of virtual larvae that enter the larval pool. Each larva can be assigned agent parameters reflecting organismal attributes (e.g., larval life duration). The model additionally encompasses the HYCOM ocean circulation module. The particle tracking module also compiles data on the number of larvae that enter the postlarval pool after re-entering a stream mouth.

Dispersal model with constant larval export: To derive the physically driven dispersal matrix, we released virtual larvae into our model from each stream mouth at a uniform level and rate throughout the study period. This involved releasing 1000 virtual larvae per day from each of the 51 stream mouth locations, with larvae at large for either 55 or 150 days. We then determined the island of origin for all successfully settled larvae and calculated settlement probabilities for all streams. This enabled us to construct a pair-wise probability matrix to illustrate the likelihood of local retention versus long distance transport. For 55 day LLD runs, a total of 43,707,000 particles were released (51 sites x 1000 particles/day x 857 days). For the 150-day LLD runs, a

total of 38,913,000 particles were released (51 sites x 1000 per site/day x 763 days). The model was run three times for each LLD scenario, with the model output averaged over all three runs.

***Dispersal model with variable larval export*:** Spawning is not constant throughout the year, and larval export is thought to be related to physical drivers such as watershed size and discharge (Cowen et al. 2003, Paris et al. 2004, Paris et al. 2005, Paris et al. 2007). We therefore evaluated a scenario where larval export was scaled to temporal and spatial variation in stream flow conditions. Stream flow data available from the Division of Aquatic Resources stream gauges (USGS - waterdata.usgs.gov/hi/nwis/sw/) in combination with stream flow data collected in the field were used to estimate monthly stream flow (cubic meters per second) for the 51 targeted watersheds. The program TableCurve 2D was used to estimate the best fit non-linear model using adjusted r^2 criteria for models with no undefined regions. Total annual larval export for each watershed was scaled to mean annual flow. Daily larval export was scaled to daily stream flow such that higher stream flow resulted in more propagules released. The total spawning outputs for all 51 sites were kept as close as possible to the constant spawning model run, totaling 44,344,172 and 39,059,525 propagules for 55 LLD and 150 LLD run respectively. These simulations were only run once per LLD scenario, therefore the results have not yet been verified through comparisons across multiple runs.

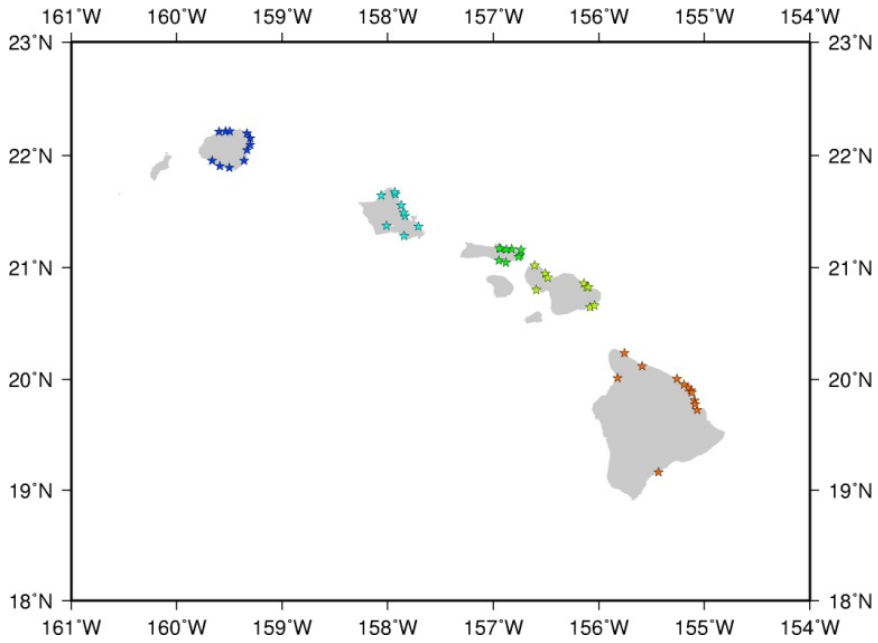

Figure 10: Stream mouth locations (stars) for release and settlement of virtual larvae across the five Hawaiian islands with perennial streams. Watersheds examined were the same as in the among-watershed assessment study described above.

4.1 Genetic and Integrative Assessment of Pacific Island Watersheds

This study was carried out to determine whether genetic measures serve as sensitive indicators of site-level and or/watershed-scale degradation, and to assess multi-scale patterns of covariance among environmental variables and measures of genetic diversity, population densities, and

species diversity. This involved comparison of mtDNA and microsatellite-based estimates of genetic variation to native and non-native species population densities, assemblage structure, water chemistry, and watershed land use. Similar comparisons were conducted to assess relationships across longitudinal transects within four watersheds. To support both studies, estimates of population densities derived from snorkel surveys were validated through comparison to estimates derived from mark-recapture approaches.

4.1.1 Among-Watershed Assessment of Environmental Condition

***Study species*:** In this study, we compared site, watershed and island level patterns of genetic variation exhibited by *Sicyopterus stimpsoni*, an endemic, moderately intolerant species capable of dispersing far inland, to patterns exhibited by *Awaous stamineus*, which is a more tolerant (putatively) endemic species common to stream reaches at lower- and middle-elevations on each island (Keith 2003, Lindstrom et al. 2012). For each species, we characterized both mitochondrial DNA haplotype diversity and nuclear microsatellite allelic diversity to assess how levels of genetic diversity vary according to biotic and abiotic stressors. Assessments were made for two separate sampling periods (2009 and 2011), and for both sampling periods combined.

Site locations, sampling design, and sample collections: To evaluate the effects of environmental stressors on native Hawaiian stream fishes, we implemented a hierarchical sampling design to account for within-watershed, between-watershed and between-island variance. This involved sampling *S. stimpsoni* and *A. stamineus* from replicate reaches within replicate watersheds of each of three land use or stewardship categories across the archipelago (Figure 6, Table 1).

Tissue samples were collected from adult and postlarval *S. stimpsoni* and *A. stamineus* from streams on Kauai, Maui, Molokai, and Hawaii. Adult and postlarval *A. stamineus* were also collected on Oahu. Adult *S. stimpsoni* were not captured on Oahu. Permit conditions prevented us from capturing adult *S. stimpsoni* on Oahu because it is now rare on the island (Burr 2001, Henderson 2003, Blum et al., unpublished data, Hawaii Watershed Atlas; http://hawaii.gov/dlnr/dar). Postlarvae of *S. stimpsoni* were occasionally sampled on Oahu due to the difficulty of species-level field identification. In accordance with historical collection records (Hawaii Watershed Atlas; http://hawaii.gov/dlnr/dar), tissues from specimens were obtained from up to 6 (Molokai) to 12 (Oahu) watersheds on each island for each species, with the exception of *S. stimpsoni* adults on Oahu (Figure 6, Table 1). Watersheds were categorized as forested, ag-urban, or military according to dominant land use or stewardship. Sets of three forested and three ag-urban watersheds were sampled on all islands except for Molokai, where four forested and two ag-urban watersheds were sampled (Figure 6). Military watersheds were only sampled on Oahu because perennial streams do not cross DoD installations on other islands (Figure 6). Supplementary samples were obtained from other watersheds for reference or analysis of historical colonization and connectivity (see section 4.0.1). Between 1 and 6 sites were sampled in each watershed in 2009 and 2011, for a total of 107 sites in 41 watersheds. Additional abiotic and biotic data (i.e., water chemistry, assemblage structure) was obtained from additional watersheds where specimens were not sampled. In total, data and/or specimens were obtained at 164 sites in 56 watersheds.

Both species were captured using hand nets. Because they are difficult to collect via hand netting, we also attempted to collect immigrating postlarvae at each location using modified Breder traps as in Benbow et al. (2004). However, trap rates were sufficiently low that we abandoned the use of Breder traps for this study. Up to 15 adults and 15 postlarvae of each species were captured per location, with the exception of *S. stimpsoni* adults on Oahu (Table 1). This enabled us to examine ≤45 adults and ≤45 postlarvae per species from each watershed (with the exception of *S. stimpsoni* on Oahu) to provide a hierarchical assessment of site-to-island scale relationships between genetic and traditional biotic indicators and environmental stressors.

Each captured specimen was kept temporarily in a holding container for subsequent processing. Stage of maturity was assessed by total length measurements. Individuals of *S. stimpsoni* of total length > 4.5cm and *A. stamineus* > 7.5cm were categorized as adults (Kinzie et al. 1984, Gingerich et al. 2005). Specimens of *S. stimpsoni* between 2.5cm and 4.5cm and *A. stamineus* between 4.5cm and 7.5cm were categorized as young-of-year juveniles. We categorized *S. stimpsoni* specimens smaller than 2.5cm and *A. stamineus* smaller than 4.5cm as immigrating postlarvae. Clips of approximately 10-30 mg of the second dorsal fin were taken from each juvenile and adult specimen and preserved immediately in 95% ethanol. Due to the difficulty of identifying postlarvae to species by morphology (Lindstrom 1999), all immigrating postlarvae were sacrificed for genetic analysis. All juveniles and adults were released after being processed except for a subset of *A. stamineus* that were sacrificed for the study of otolith microchemistry (see section 4.0.2).

Water chemistry: At each sampling event, water samples were filtered and stored frozen for later analysis of soluble reactive phosphorus (SRP), total phosphorus (TP), nitrate (NO_3), ammonium (NH_4), and total dissolved nitrogen (TDN) using standard colorimetric methods. Total suspended sediments (TSS) were measured by filtering stream water through pre-weighed filters (ProWeigh 1.5 μm pore size), drying filters at 60˚ C for 48 hrs, and reweighing the dried filters. Total dissolved solids (TDS) were measured using a HM hand-held meter.

PCA was used to derive axes summarizing major patterns in water chemistry, accounting for spatial correlations among the measured variables. With substantial spatial covariance among water quality parameters, PCA creates axes that describe the maximum possible variability in the data set, eliminating the need for statistical tests of each individual parameter and associated statistical problems with multiple comparisons. This analysis was conducted separately for 2009 and 2011 water chemistry data.

We also assayed primary producers, snails, and a native goby (*Awaous stamineus*) for nitrogen stable isotopes. Ratios of nitrogen stable isotopes (^{15}N:^{14}N, expressed as $\delta^{15}N$) were measured to provide an integrative measure of anthropogenic disturbance of nutrient cycling. Nitrogen isotopes are highly sensitive tracers of nutrient sources, and have been widely used to distinguish inputs from sewage, agriculture, and other human activities (Fry 1999, Schlacher 2005). Rather than solely relying on measures of isotopic nitrogen in solution or primary uptake compartments (algae, microbes), we also sampled animal tissues because they integrate isotopic patterns over periods of months to years (Post 2002). By sampling algae as well as muscle tissue from primary consumers (gastropods) and secondary consumers (*A. stamineus*), we were able to assess shifts in nitrogen sources contributing to both consumer trophic levels in Hawaiian stream food webs

(Sorenson and Hobson 2005). Where possible, five or more replicate samples per trophic level were sampled from each sample site in 2009 and 2011. Samples were dried, ground to a fine powder, and 1 milligram (mg) subsampled for analysis using a Finnigan MAT Delta-Plus mass spectrometer at the Cornell University Stable Isotope Laboratory. Values of $\delta^{15}N$ were calculated based on comparisons across trophic groups as a measure of nutrient loading at each site (Post 2002). We also compared values of $\delta^{15}N$ with land use patterns assessed from satellite imagery and water chemistry parameters, with the expectation that nitrogen isotopes of the stream fauna offer a sensitive, in-stream indicator of human activities and nutrient loading in each watershed.

Additionally, we compared stable isotope ratios between *A. stamineus* and primary consumers (grazing snails) to evaluate the trophic status of *A. stamineus* across a range of water quality conditions (Post 2002). *Awaous stamineus* is considered to be omnivorous, as it feeds primarily on green algae and aquatic insects from the benthos and water column (Kido 1997), but it is possible that the species occupies a different ecological niche under degraded stream conditions. From Post (2002), trophic position (T) of *A. stamineus* (T_{AS}) was calculated as:

$$T_{AS} = (\delta^{15}N_{AS} - \delta^{15}N_{snail})/3.4 + 2$$

Watershed land use: Land use data for each watershed was derived from the 30-meter resolution 2001 National Land Cover Dataset (NLCD) (Homer et al. 2007). Data were extracted for each spatial assessment unit using ArcGIS workstation version 9.3 (ESRI). Though data were extracted for fifteen different land use categories, we focused our analyses on the percentage of the landscape classified as forested (%forested) and the percentage of the landscape classified as agricultural and urban land use (%ag-urb). Our %forested metric reflects the evergreen forest classification category in the NLCD. Our %ag-urb metric reflects an additive combination of land cover classified as either developed (open space, low, medium, or high intensity), pasture/hay, or cultivated crops (Homer et al. 2007).

PCA also was used to derive axes summarizing major patterns in land cover to account for spatial correlations among the measured variables. With substantial spatial covariance among classifications, PCA created axes that describe the maximum possible variability in the data set, complementing use of targeted individual parameters for statistical analyses of relationships between genetic (and related) indicators, in-stream biotic and abiotic conditions, and land cover / land use conditions.

All land cover metrics were determined at the site level and watershed scale. Watershed boundaries and watershed catchment areas were identified or calculated from the National Hydrography Dataset Plus (NHD+) (McKay et al. 2012), also using ArcGIS (ArcGIS Desktop: Release 9.3, ESRI). Metrics were determined at the site level using catchment boundaries that correspond to the land surface that drains directly into a NHD+ flow-line without first flowing into an upstream flow-line (Homer et al. 2007). Catchment boundaries were delineated for each sample site using Arc Hydro tools version 1.4.

Population and community surveys: Following standardized methods (Higashi and Nishimoto 2007), point-quadrat visual surveys were conducted at each sampling event to estimate native and non-native fish and invertebrate densities and distributions. The point-quadrat method

involves snorkelers counting individuals of each resident species in 30 randomly selected, one m^2 quadrats distributed across a 100 m long section of stream (Baker and Foster 1992). Population density estimates of each species present in the surveyed quadrats were calculated as the number of fish or invertebrates recorded in quadrats divided by the area sampled.

***DNA extraction and mitochondrial DNA sequencing*:** Genomic DNA was recovered from fin tissue or lateral muscle samples (from specimens sacrificed for otolith analysis) for both species using DNeasy (Qiagen, Valencia, CA) extraction kits designed for animal tissue.

For all *Awaous stamineus*, a ~ 500 bp section of the mitochondrial cytb gene was amplified using approximately 10 ηg of template DNA and two primers, GluFish (5'–ACCACCGTTGTTATTCAACTACAA–3'; and ThrFish2, 5'–AACCTCC GACATCCGGCTTACAAGACCG–3') in a 15 ml PCR cocktail subjected to thermal cycling conditions consisting of an initial denaturation step at 94^{o}C for 6 min, followed by 35 cycles of 94^{o}C for 15 s, 52^{o}C for 15 s, 72^{o}C for 15 s, and a final extension at 72^{o}C for 10 min using MBS Satellite 0.2G thermo cyclers (Thermo Electron Corporation). PCR amplicons were purified using ExoSAP-It (USB, Affymetrix) and cycle sequencing reactions were performed using FishSeq (5'–CCACCGTTGTTATTCAACTAC AAG–3') and ThrFish2 primers and BigDye 3.1 chemistry (Applied Biosystems). All sequencing reactions were purified using Sephadex (GE Healthcare Biosciences) plate protocols and analyzed on an ABI 3100 or ABI 3730xl automated DNA sequencer (Applied Biosystems).

Due to the absence of informative levels of haplotype and sequence variation, DNA sequence data was not collected for *Sicyopterus stimpsoni* in this study.

Awaous microsatellite genotyping: Tissue clips also were used to genotype all *A. stamineus* at 13 microsatellite loci. Nine of the loci that were used have been published in Hogan et al. (2010): Agua A4, Agua B1, Agua B2, Agua C4, Agua D3, Agua D9, Agua D103, Agua D110, and Agua D135. The remaining loci that were used are Agua D106, Agua D117, Agua D6, and Agua D7 (see section 4.0.1).

PCRs were performed in 15 µL volumes using MBS Satellite 0.2G thermo cyclers (Thermo Electron Corporation), with conditions following Hogan et al. (2010). HEX, 6-FAM or NED fluorescently dye-labeled forward primers were used to generate labeled PCR amplicons for sizing the loci against a 500 ROX™ or LIZ™ size standard on an ABI 3100 or ABI 3730xl DNA analyzer. Electropherograms were scored using GENEMARKER v1.9 (SoftGenetics LLC).

Sicyopterus microsatellite genotyping: Tissue clips were used to genotype *S. stimpsoni* at the 12 microsatellite loci described in Table 2. Methods for amplifying, sizing and scoring of these loci were identical to those followed for microsatellite genotyping of *A. stamineus* as described above, with the exception of locus-specific annealing temperatures (Table 2).

Awaous mitochondrial DNA analysis: All raw sequence files were edited, assembled, and aligned with Sequencher v4.9 (Gene Codes Corp., Ann Arbor, MI). Nucleotide composition of the sequenced cytb region was examined for variable sites with Sequencher v4.9 and GENALEX v6.5. Identification of unique haplotypes and haplotype frequencies at each sample location was

performed in TCS 1.21. ARLEQUIN v3.1 was used to estimate haplotype diversity, nucleotide diversity, and effective haplotype numbers for each population. Effective haplotype numbers were calculated as the reciprocal of the summation of the squared haplotype frequencies at that locality. ARLEQUIN v3.1 also was used to perform spatial and temporal hierarchical AMOVAs to determine the extent to which variation in haplotype diversity was attributable to differences among sites and among sample years.

***Microsatellite data analysis*:** A suite of genetic diversity indices were estimated for all sites for both *A. stamineus* and *S. stimpsoni*. MSA v4.05 was used to calculate expected heterozygosity, Shannon diversity index values, and the number of alleles per locus. MSA v4.05 was also used to calculate rarified allelic richness for each site. ARLEQUIN v3.1 was used to identify departures from Hardy-Weinberg equilibrium (HWE) for each locus, and to perform spatial and temporal hierarchical AMOVAs to determine the extent to which genetic variation was attributable to differences among sites.

Statistical analysis: For both species, Pearson correlation coefficients were first calculated to assess the strength of pair-wise associations between measures of genetic diversity, population density, and species richness (Vellend 2004, Blum et al. 2012). We then assessed pair-wise associations between each of these measures and measures of biotic (e.g., native species richness, non-native species densities) and abiotic conditions (e.g., water chemistry, elevation, distance to mouth) at each site. Additionally, analysis of variance (ANOVA) was conducted to determine the presence of significant differences in measures of diversity and population densities among islands and land use categories. Analyses were run separately at the site and watershed scale.

Forward stepwise regression was used to evaluate how variation in genetic diversity, population density, and species richness correspond to categorical sets of biotic (e.g., non-native species richness and densities) and abiotic variables (e.g., PC factors for water chemistry, %ag-urb land cover), where the number of variables was first reduced by eliminating collinear variables and significant co-variants (Blum et al. 2012). We used $p = 0.15$ as the significance level for inclusion and exclusion of terms in regression models (Vellend 2004, Blum et al. 2012). All statistical analyses were conducted using SYSTAT 13 (SPSS).

4.1.2 Within-Watershed Assessment of Environmental Condition

***Study species*:** We chose to examine *Awaous stamineus* to test hypotheses of within-watershed variation in demography and genetic diversity. *A. stamineus* was chosen because it is a culturally important species and because it exhibits high population densities across long elevational gradients, even in streams suffering from moderate anthropogenic degradation. Though *A. stamineus* have the ability to climb (Kinzie 1988, Keith 2003), the species is generally limited to reaches at lower and intermediate elevations that occur below the first major watershed barrier (e.g., a high waterfall). We also chose to examine *A. stamineus* because it co-occurs and/or potentially interacts with a range of non-native species including some of the most common non-native species, like Poeciliid guppies and other livebearers (Eldredge 2000, Yamamoto and Tagawa 2000, Brasher 2006).

Site locations, sampling design, and sample collections: Sample sites were located in three watersheds along the Hamakua Coast on the island of Hawaii and one watershed on the north shore of Oahu. The Hamakua coast encompasses a series of similarly sized watersheds distributed across a gradient of land use and fish assemblage structure. The three Hamakua watersheds chosen for this study exhibited little (Hiilawe), moderate (Hanawi), and heavy (Maili) ag-urban land use development (Figure 11). While all three watersheds supported high densities of native fishes, only Maili contained Poeciliids at all three sites. No Poeciliids were observed at any site in Hanawi, and Poeciliids were only observed at the lowest site at Hiilawe. A portion of the watershed on Oahu (Waimea), which exhibits moderate ag-urban development and supports low densities of native species, is under military stewardship. The majority of the middle and upper watershed falls within Kawailoa Training Area, which has supported Stryker Brigade training operations.

Data on in-stream conditions and *A. stamineus* tissue samples were collected in 2010-2011 from sites longitudinally distributed in each watershed. Three of the sites in each watershed corresponded to locations where individual mark-recapture surveys were being carried out (see section 4.1.3). Surveys were conducted at one low, one mid and one high site within each watershed. Chosen sites were between zero and 2.7 km from the stream mouth, with pairwise distances ranging from 0.07 km to 2.56 km apart (see section 4.1.3 for a more detailed description of the study sites). Tissues were collected from three additional sites within each watershed, with supplemental sites interspersed among the mark-recapture survey sites.

The number of specimens that were examined differed between mark-recapture and supplemental sites. At each site, individual *A. stamineus* were caught with hand nets. Only a single capture event occurred at each supplemental site, whereas six or seven capture events occurred for each mark-recapture site between June 2010 and March 2011. Each individual captured at a mark-recapture site was given an individual mark (see section 4.1.3). Weight,

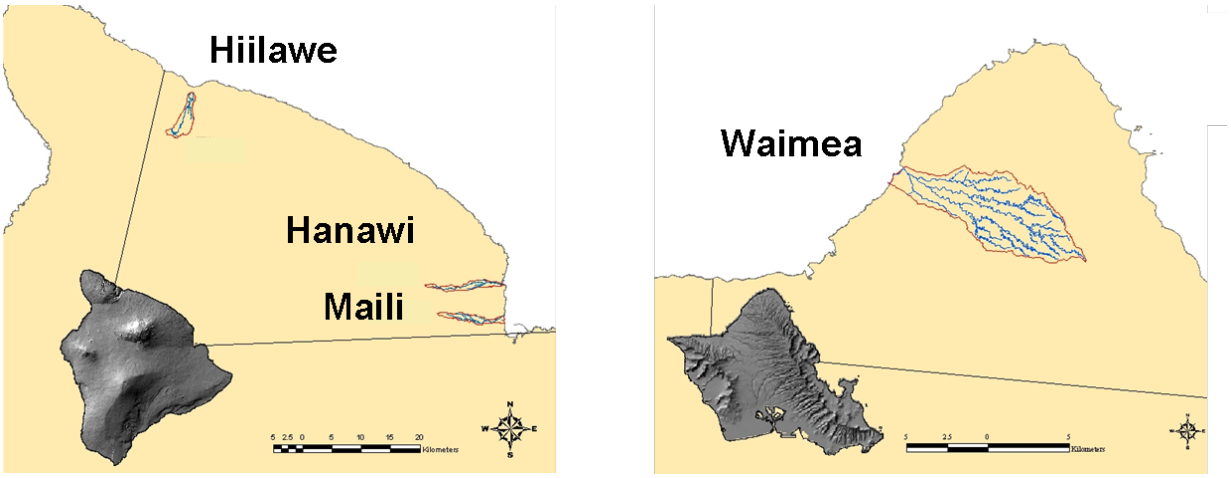

Figure 11: The location of individual mark-recapture study watersheds on the north shore of Oahu and along the Hamakua coast on the island of Hawaii.

length, and overall condition also were recorded for each fish captured at these sites. Tissue clips of approximately 10-30 mg were taken from the second dorsal fin of each newly captured fish at all sites. Fin clips were preserved in 95% ethanol. Between 57 and 201 individuals were examined at mark-recapture sites, and between 11 and 51 individuals were examined at supplemental sites. In total, 1,354 tissue samples were collected from 22 sites.

Population surveys: Following standardized methods (Higashi and Nishimoto 2007), point-quadrat visual surveys were conducted at each site to estimate fish and invertebrate densities and distributions. The point-quadrat method involves snorkelers counting residents in 30 randomly selected, 1 m^2 quadrats distributed across a 100 m long section of stream (Baker and Foster 1992). Population density estimates of each species present in the surveyed quadrats were calculated as the number of fish recorded in quadrats divided by the area sampled. Visual surveys were conducted immediately prior to capture events at sites where mark-recapture surveys were conducted.

Additional demographic parameters were estimated for individual capture events and across all capture events at sites where mark-recapture surveys were carried out. Estimates were calculated in the program MARK using the POPAN parameterization (White and Burnham 1999). Model parameters were selected to allow for survival and capture probability to vary between the dry season sampling period (June-October) and the wet season sampling period (November-March). Apparent recruitment and survival were also calculated using the Pradel model parameterized to allow for seasonal differences. ANOVA was used to determine significant differences in apparent recruitment and survival among watersheds and among sites within watersheds. Growth coefficients and theoretical asymptotic maximum lengths were characterized for each site using the von Bertalanffy growth curve (von Bertalanffy 1938).

Water chemistry: Water chemistry was measured once at each site. Water samples were collected from the thalweg, filtered immediately (Whatman GFX, 0.45 µm pore size), and stored frozen until analysis for soluble reactive phosphate, ammonium, nitrate and total nitrogen by standard colorimetric methods (see section 4.1.1).

***DNA extraction and microsatellite genotyping*:** Tissue clips were used to genotype *A. stamineus* at 13 microsatellite loci. Nine of the loci that were used have been published in Hogan et al. (2010): Agua A4, Agua B1, Agua B2, Agua C4, Agua D3, Agua D9, Agua D103, Agua D110, and Agua D135. The remaining loci that were used were Agua D106, Agua D117, Agua D6, and Agua D7 (see section 4.0.1).

Genomic DNA was extracted from fin clips using a DNeasy tissue extraction kit (Qiagen). PCRs were performed in 15 µL volumes using MBS Satellite 0.2G thermo cyclers (Thermo Electron Corporation) with conditions following Hogan et al. (2010). HEX, 6-FAM or NED fluorescently dye-labeled forward primers were used to generate labeled PCR amplicons for sizing the loci against a 500 ROX™ or LIZ™ size standard on an ABI 3100 or ABI 3730xl DNA analyzer. Electropherograms were scored using GENEMARKER v1.9 (SoftGenetics LLC).

***Microsatellite data analysis*:** A suite of genetic diversity indices were estimated for all sites. Observed and expected heterozygosity, Shannon diversity index values, and private allele

frequencies were calculated using GENALEX v6.5. MSA v4.05 was used to calculate the number of alleles per locus and rarified allelic richness for each site. ARLEQUIN v3.1 was used to identify departures from HWE for each locus, and to perform spatial and temporal hierarchical AMOVAs.

We also estimated patterns of genetic differentiation among sites within each watershed. MSA v4.05 was used to assess population subdivision by calculating pairwise F_{ST} values, with values bootstrapped over 999 replications (Slatkin 1995). To test for isolation by distance (IBD) within each watershed, we compared a matrix of pairwise F_{ST} values with a corresponding pairwise matrix of between-site river kilometers. Genetic autocorrelations with geographical distance was evaluated in GENALEX v6.5 by creating correlograms of Mantel's *r* versus river kilometers for each watershed (Mantel 1967). We further examined population structure within each watershed using STRUCTURE v2.1, which applies a Bayesian clustering algorithm to assign individuals to groups by minimizing deviations from linkage disequilibrium. After a burn-in period of 30,000 MCMC iterations, we collected data from an additional 5×10^5 iterations for 3 replicate sets of runs with *k* iteratively set from 1 to 6. Data for each watershed were run separately. We parameterized all runs under conditions of admixture and with allele frequencies considered independent between populations. We identified the number of populations in each watershed based upon the greatest P(X|K) value and the break in the slope of the distribution of P(X|K) values, which is a good predictor of the uppermost hierarchical level of genetic structure among sampled individuals (Evanno et al. 2005).

We used NeEstimator v1.3 (Ovenden et al. 2007) to calculate estimates of effective population size (N_e) for sites and watersheds according to linkage disequilibrium method based on Burrow's composite measure of disequilibrium as well as the molecular co-ancestry method (Campton 1987, Bartley et al. 1992, Ovenden et al. 2007, Waples and Do 2008).

Statistical analysis: Pearson correlation coefficients were calculated to assess the strength of pair-wise associations between measures of genetic diversity, N_e, and *A. stamineus* population density. We then assessed pair-wise associations between these measures and measures of biotic (e.g., native species richness, non-native species densities) and abiotic conditions (e.g., water chemistry, elevation, distance to mouth) at each site. Analyses were run separately for all three watersheds and cumulatively across all watersheds (Vellend 2004, Blum et al. 2012).

Stepwise forward regression was used to evaluate how measures of diversity and *A. stamineus* population density correspond to categorical sets of biotic and abiotic environmental variables, where the number of variables was reduced by elimination of collinear variables and significant co-variants (see section 4.1.1; Vellend 2004, Blum et al. 2012). General linear modeling was also used to evaluate the extent to which variation was attributable to physiographic conditions and watershed-scale differences. All statistics were calculated using SAS v9.3 software (SAS Institute 2012).

4.1.3 Mark-recapture Calibration of Snorkel Surveys

Study species: This work focused on *Awaous stamineus.* Like the other four stream fish endemic to the Hawaiian Islands, *A. stamineous* exhibits a benthic habit as an adult. Hawaiian streams are

also home to a rich assemblage of non-native fishes that co-occur with native species. Some of the most common non-native species, like Poeciliids (e.g., guppies and other livebearers), occur at such high densities that their presence may contribute to observer bias in visual surveys. Calibration studies of visual surveys can potential identify and correct for such biases.

Individual mark-recapture: We conducted individual mark-recapture of adult *A. stamineus* (TL > 40 mm) at twelve sites in four watersheds. Three of the watersheds were on the island of Hawaii, and one watershed was on the island of Oahu (Figure 11, Table 4). Sites were chosen to represent a gradient in land use and fish communities. The three watersheds on Hawaii exhibit little (Hiilawe), moderate (Hanawi), and heavy (Maili) ag-urban development (Figure 11). While all three watersheds supported high densities of native fishes, only Maili contained Poeciliids at all three sites. No Poeciliids were observed at any site in Hanawi, and Poeciliids were only observed at the lowest site at Hiilawe. A portion of the watershed on Oahu (Waimea), which exhibits moderate ag-urban development and supports low densities of native species, is under military stewardship. The majority of the middle and upper watershed falls within Kawailoa Training Area, which has supported Stryker Brigade training operations. Poeciliids and other non-native fishes were observed at all sites in the watershed.

One low, one mid and one high site was examined within each watershed. Low sites represented the lowest part of the watershed where use of hand nets was feasible (i.e., due to stream width and depth, as well as water clarity). High sites were located just downstream of the first major barrier preventing further upstream dispersal of *A. stamineus.* Mid sites were located between high and low sites, but above smaller barriers preventing further upstream dispersal of the predatory *Eleotris sandwicensis.* Chosen sites were between zero and 2.7 km from the stream mouth, with pairwise distances ranging from 0.07 km to 2.56 km apart. All sites contained a mixture of runs, pools, and riffles habitats generally representative of the whole stream.

At each site, individual *A. stamineus* were caught with hand nets, temporarily anesthetized with Tricaine methanosulfonate (MS-222) and given individual elastomer marks. All marks were visible implant elastomer tags (VIE), a pliable and biologically inert product produced by Northwest Marine Technology, Inc. Tags were inserted just beneath the skin using a 29 gauge syringe and needle. Each fish received 3 marks, each in one of 10 different mark locations, using a combination of up to three different colors. Weight, total length, and overall condition were recorded for each captured individual. Individuals were then placed in well aerated holding containers to recover and then returned to their original position in the stream. Capture events were conducted six or seven times at each site on Hawaii, and three times on Oahu, from June 2010 to March 2011 (Table 4). Approximately 30,000 fish were encountered and 2,850 *A. stamineus* individuals were sighted or marked across all sites and sampling events (Table 4).

Demographic parameters for each site from individual recapture events and across all samples were calculated using the program MARK following POPAN parameterization (White and Burnham 1999). Model parameters were selected to allow survival and capture probability to vary between the dry season sampling period (June-October) and the wet season sampling period (November-March). Apparent recruitment and survival were also calculated using the Pradel model parameterized to allow for seasonal differences (dry/wet). ANOVA was used to determine significant differences in apparent recruitment and survival among watersheds and among sites

within watersheds. Growth coefficients and theoretical asymptotic maximum lengths were characterized for each site using the von Bertalanffy growth curve.

Point-quadrat visual surveys were conducted at each site immediately prior to capture events. The standard method of assessing abundance and distribution estimates of stream fish in Hawaii is a point-quadrat visual survey (Higashi and Nishimoto 2007). The point-quadrat method involves snorkelers counting resident fish in 30 randomly selected quadrats distributed across a 100 m long section of stream. Quadrats were no larger than 1 m^2 to avoid undercounting individuals in smaller size classes (Baker and Foster 1992). Population density estimates of each species present in the surveyed quadrats were calculated as the number of fish recorded in quadrats divided by the area sampled.

Batch mark-recapture: We also estimated population sizes via batch mark-recapture of adult *A. stamineus* (TL > 40 mm) at one site within each of the watersheds where the individual mark-recapture study was conducted (Table 4) between June 2010 and March 2011. We also estimated population sizes at 13 additional sites across the Hawaiian archipelago (on all islands except Kauai due to inclement weather) from March to July 2011 (Table 4). At each site, individual fish were captured immediately following a visual survey. Captured fish were temporarily anesthetized with MS-222 and given a batch elastomer mark. Batch marking involved use of one color and location. Weight, total length, and overall condition were recorded for each captured individual. Individuals were then placed in well aerated holding containers to recover and then returned to their original position in the stream. Recaptures were conducted the following day.

Population densities for each site from batch mark-recapture events were calculated using the Lincoln-Peterson estimator.

Calibration analysis: We used geometric mean (GM) regression analysis to compare density estimates between methods. When the predictor variable is known to contain error, error-in-variables regression techniques such as GM regression (also called reduced major axis regression) produce more meaningful descriptions of the relationship between variables than least-squares linear regression (McArdle 2003). We fixed all regression intercepts to zero so that the regression slope could be compared to the 1:1 line. All population density estimates were transformed as log (x+1) prior to analysis to make their variances homoscedastic. Kolmogorov–Smirnov tests were used to compare estimates of size structure between visual surveys and individual mark-recapture methods. The two-sample Kolmogorov-Smirnov test is a nonparametric test that compares two cumulative frequency distributions and reports the probability that there is no difference between classes or samples.

We also tested for potential covariates that might affect the densities and size structures estimated from visual surveys and mark-recapture approaches. For densities, we used the y-axis residuals from the GM regressions as the response variable in a general linear model that included watershed land use, Poeciliid presence, stream position, site, and capture event. We used the Waller-Duncan Multiple Comparison Procedure to group significantly different covariates. Kolmogorov-Smirnov tests were used to test whether size estimates were significantly different with respect to potential covariates. All statistics were calculated using SAS 9.3 software (SAS Institute 2012).

Site	Island	Visual Survey		Batch Mark-Recapture				Individual Mark-Recapture		
		Events	No	Events	Nm	Nc	Nr	Events	Ntm	Ntr
Hiilawe-Low	Hawaii	7	30					7	115	95
Hiilawe-Mid	Hawaii	6	71					6	240	260
Hiilawe-High	Hawaii	6	22	1	18	16	12	7	141	156
Hanawi-Low	Hawaii	7	22					7	71	70
Hanawi-Mid	Hawaii	6	12	1	37	26	21	6	109	104
Hanawi-High	Hawaii	7	57					7	206	122
Maili-Low	Hawaii	7	71	1	65	32	15	7	294	163
Maili-Mid	Hawaii	7	62					7	227	73
Maili-High	Hawaii	7	47					7	171	106
Waipio-High	Hawaii	1	8	1	35	35	5			
Hakalau-Low	Hawaii	1	3	1	20	13	2			
Alelele-Low	Maui	1	44	1	76	56	21			
Waihee-Low	Maui	1	8	1	33	27	8			
Honokohau-Low	Maui	1	3	1	31	29	9			
Halawa-Mid	Molokai	1	28	1	121	121	77			
Honouli Wai-High	Molokai	1	13	1	30	24	9			
Keaahala-Mid	Oahu	1	75	1	69	84	12			
Kahana-Mid	Oahu	1	7	1	37	35	11			
Waimea-Low	Oahu	2	4					3	13	4
Waimea-Mid	Oahu	3	3					3	23	6
Waimea-High	Oahu	3	8	1	9	7	1	3	56	10

Table 4: Numbers of individuals encountered in visual surveys (No = number of observed fish), batch mark-recapture (Nm = number of fish marked; Nc = number of captured fish; Nr = number of recaptured fish), and individual mark-recapture (Ntm = number of total marked fish; Ntr = number of total recaptured fish) per site. See Figure 9 for location of individual mark-recapture watersheds.

5 Results and Discussion

5.0 Historical Colonization and Contemporary Connectivity

5.0.1 Genetic Analysis of Historical Colonization

Genetic variation in Awaous: Sequence data for a 512bp region of the mtDNA cytb gene was obtained for a total of 2237 *Awaous stamineus* sampled from 41 watersheds in 2009 and 2011 (Table 1). A cumulative total of 101 distinct haplotypes were recovered among the sampled individuals differentiated at 75 polymorphic sites. A total of 49 mtDNA haplotypes were recovered from 1030 individuals sampled in 2009. A total of 83 mtDNA haplotypes were recovered from 1219 specimens sampled in 2011, with 52 of those haplotypes being distinct from those recovered in 2009. In a given year, the number of mtDNA haplotypes per watershed

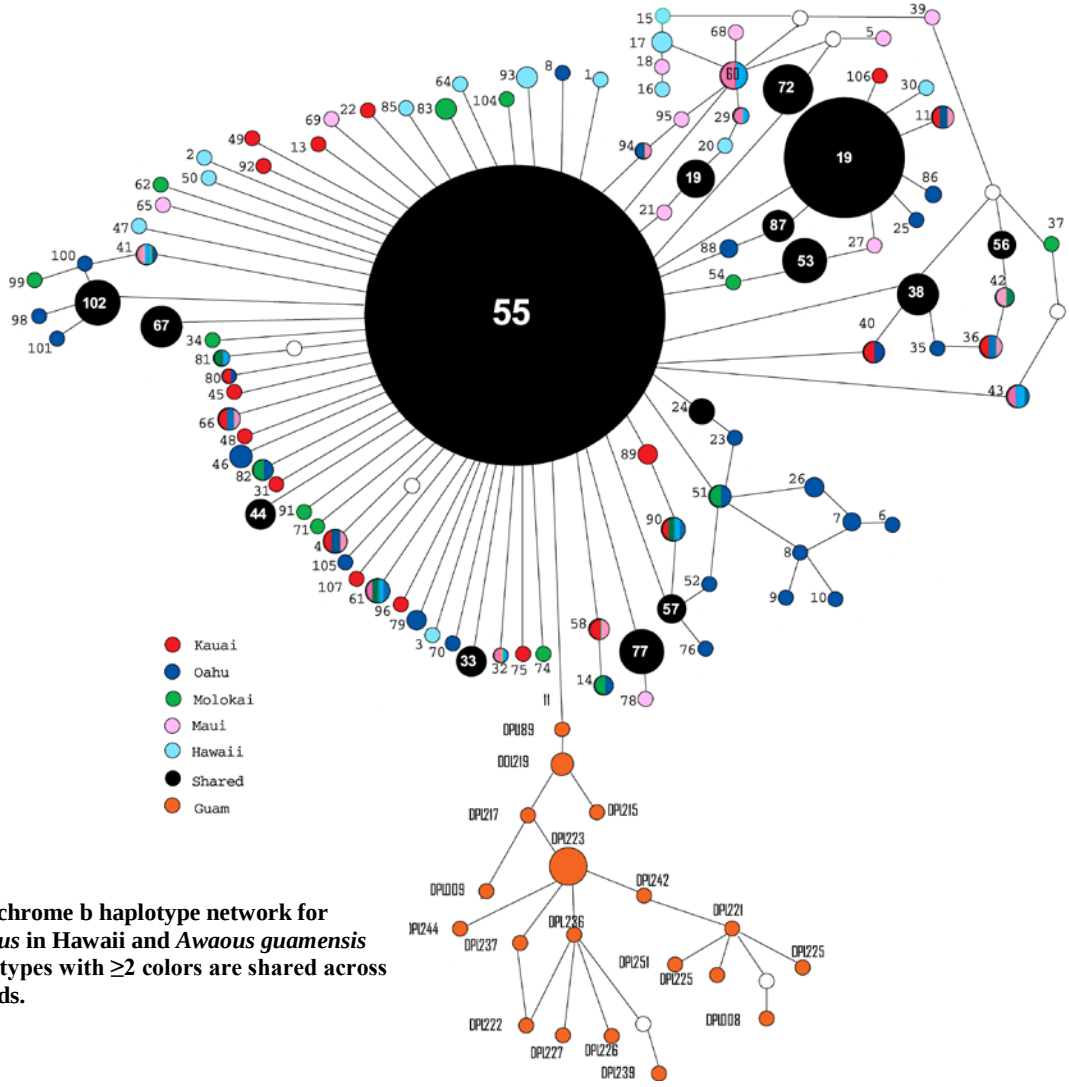

Figure 12: Cytochrome b haplotype network for *Awaous stamineus* in Hawaii and *Awaous guamensis* in Guam. Haplotypes with ≥2 colors are shared across a subset of islands.

varied between 1 and 15, with cumulative values ranging as high as 20 per watershed. The number of mtDNA haplotypes per island ranged between 12 and 26 in 2009 and from 23 to 42 in 2011, with cumulative values ranging from 34 to 51 haplotypes per island over both years (Table 5). Haplotype diversity was 0.501 in 2009 and 0.576 in 2011. Cumulative haplotype diversity over both years was 0.542. Cumulative nucleotide diversity over both years was 0.00144.

The majority of haplotypes differed by only a single base pair substitution, though the amount of divergence ranged as high as 15 substitutions, or 2.9% sequence divergence, among the observed haplotypes. No distinct haplotype groups were recovered, but clustering of island-specific haplotypes was found across the reconstructed haplotype network (Figure 12). Geographical clustering was more pervasive for haplotypes recovered on Oahu and Maui than other islands (Figure 12). No bootstrap values greater than 50% were recovered for nodes across the reconstructed haplotype network, which is largely a reflection of the low pair-wise sequence divergence observed among individual cytb haplotypes.

The prevalence and distribution of *A. stamineus* cytb haplotypes were unequal across the archipelago. Though 15 haplotypes were recovered on all islands, 2 haplotypes were overwhelmingly more prevalent than all other observed haplotypes. The most common haplotype was recovered in 66.4% of individuals, whereas the second most common haplotypes was recovered in 12.7% of individuals. Another 25 less common haplotypes were shared across two or more islands. Shared haplotypes were most often recovered from neighboring islands, but disjunct distributions were found for some of the shared haplotypes. For example, 3 haplotypes were shared across Kauai, Oahu and Maui, and 1 haplotype was found on Kauai, Oahu and Hawaii. A pattern of asymmetric abundance was often observed for shared haplotypes, where a given haplotype was more prevalent on one island or a subset of islands rather than being equally abundant on all of the islands where it was found (Figure 13). Nearly all other haplotypes were rare and recovered within and among watersheds on a single island.

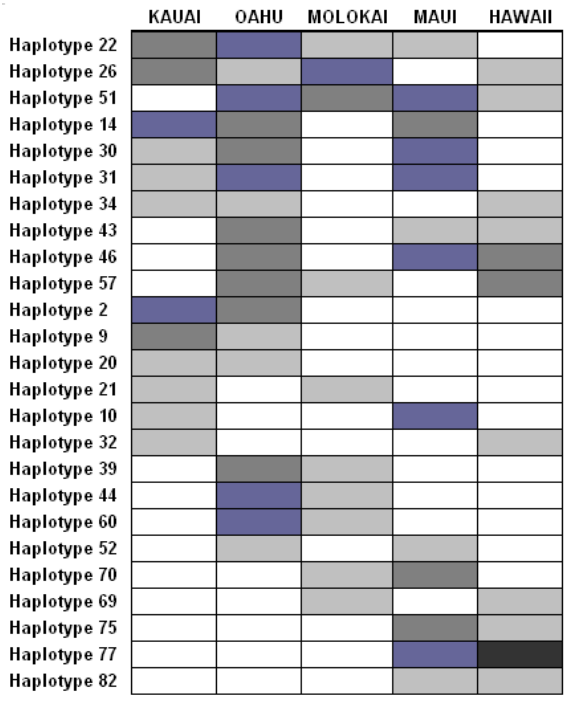

Figure 13: Occurrence and relative prevalence of shared cytb haplotypes in *Awaous stamineus*; Black = >5 individuals; Purple =≥3 individuals; Dark grey = 2 individuals; Light grey = 1 individual.

The number and proportion of common, shared and unique haplotypes varied among islands. Though the total number of common haplotypes was identical across all islands, the proportion of common haplotypes relative to all other haplotypes ranged from a low of 29.41% on Oahu to a high of 44.12% on Molokai (Table 5). The proportion of haplotypes shared across two or more islands ranged from a low of 28.21% on Hawaii to 34.29% on Kauai. The proportion of unique haplotypes on each island ranged from a low of 22.86% on Kauai to a high of 37.25% on Oahu.

Nearly all estimates of haplotype diversity were highest on Maui and Oahu (Table 5). More haplotypes were recovered on Oahu and Maui than other islands, with Oahu harboring the highest number of haplotypes. Similarly, more shared haplotypes were found on Oahu and Maui, with Oahu harboring more shared haplotypes than any other island. Haplotype diversity was highest on Maui, as was nucleotide diversity and the effective number of haplotypes (Table 5). The mean number of pairwise differences among haplotypes was also greatest on Maui and Oahu. The lowest values of pairwise differences occurred on Molokai and Kauai (Table 5).

	Kauai	Oahu	Molokai	Maui	Hawaii
Total	35	51	34	40	39
Common	15	15	15	15	15
Shared	12	17	10	13	11
Shared (4)	2	3	3	2	2
Shared (3)	4	7	1	5	4
Shared (2)	6	7	6	6	5
Unique	8	19	9	12	13
%Common	42.86	29.41	44.12	37.50	38.46
%Shared	34.29	33.33	29.41	32.50	28.21
%Shared (4)	5.71	5.88	8.82	5.00	5.13
%Shared (3)	11.43	13.73	2.94	12.50	10.26
%Shared (2)	17.14	13.73	17.65	15.00	12.82
%Unique	22.86	37.25	26.47	30.00	33.33
Hap D	0.537	0.526	0.523	0.584	0.541
Pairwise D	0.677	0.734	0.705	0.816	0.727
Nucleotide D	0.001	0.001	0.001	0.002	0.001
K	2.155	2.104	2.090	2.397	2.169

Table 5: The number and diversity of *Awaous stamineus* mtDNA haplotypes recovered on Kauai, Oahu, Molokai, Maui and Hawaii. Common = haplotype occurs on all islands; Shared = haplotype occurs on a subset of islands; Shared (4/3/2) = haplotype occurs on 4, 3, or 2 islands; Unique = haplotype only occurs on one island. Hap D = haplotype diversity on an island; Pairwise D = pairwise differences among haplotypes on an island; Nucleotide D = nucleotide diversity among haplotypes on an island; k = effective number of haplotypes on an island.

unequal distributions of haplotypes translated to significant levels of genetic differentiation among islands. Global Φ_{ST} estimates ranged from 0.003 in 2009 to 0.028 in 2011, amounting to a cumulative estimate of 0.011 across both years ($p < 0.001$). Approximately 0.21% of observed variation was attributable to haplotype frequency differences among islands across both years ($p < 0.001$). The range of pair-wise estimates of Φ_{ST} varied among years (Table 6). Consistent with the low estimated global Φ_{ST} value, pair-wise values of Φ_{ST} were low and predominantly not significant for samples taken in 2009. Only Oahu and Maui were significantly different in 2009. In contrast, pair-wise values of Φ_{ST} were consistently higher for samples taken in 2011. With some exceptions, a general pattern of increasing genetic differentiation occurred with increasing distance among islands. Nearly all pair-wise comparisons also were significant ($p < 0.05$) or nearly so ($p < 0.1$; Table 6) for 2011. Estimates based on both years recovered a consistent pattern of significant differentiation between Hawaii and all other islands. Significant pair-wise differentiation was also recovered between Oahu and Maui, as well as between Molokai and Maui (Table 6).

2009

	Kauai	Oahu	Molokai	Maui	Hawaii
Kauai	0.0000				
Oahu	0.0006	0.0000			
Molokai	-0.0010	0.0001	0.0000		
Maui	-0.0026	**0.0046**	0.0008	0.0000	
Hawaii	-0.0032	-0.0002	-0.0022	-0.0035	0.0000

2011

	Kauai	Oahu	Molokai	Maui	Hawaii
Kauai	0				
Oahu	**0.00239**	0			
Molokai	0.00178	**0.0052**	0		
Maui	**0.00273**	**0.00444**	**0.00476**	0	
Hawaii	**0.00828**	**0.01055**	**0.0074**	**0.00277**	0

Combined

	Kauai	Oahu	Molokai	Maui	Hawaii
Kauai	0				
Oahu	0.00066	0			
Molokai	0.00086	0.00062	0		
Maui	0.00007	**0.00201**	**0.00269**	0	
Hawaii	**0.00454**	**0.0045**	**0.00393**	**0.00255**	0

Table 6: Pair-wise values of Φ_{ST} based on cytochrome b haplotype variation in *Awaous stamineus* among islands. Values in bold are significant ($p < 0.05$), and values in blue are nearly significant ($p < 0.1$).

Tests of demographic and spatial expansion provide evidence of demographic but not spatial expansion in *Awaous stamineus*. The recovery of a negative Tajima's D value (-2.44), and mismatch distribution tests for the full archipelago indicate that the hypothesis of demographic expansion cannot be rejected, where an excess of low-frequency haplotype variants suggests that *A. stamineus* has undergone a recent population expansion in the archipelago. The hypothesis of spatial expansion across the archipelago can be rejected. Similarly, mismatch distribution tests indicate that the hypothesis of demographic expansion cannot be rejected for Oahu. Tests for other islands do not support the hypothesis for spatial or demographic expansion.

Genetic variation in Sicyopterus: In an exploratory study, sequence data for a 430 bp region of the mtDNA cytb gene was obtained for 111 specimens of *Sicyopterus stimpsoni* sampled from four watersheds on Hawaii (N = 28), Maui (N = 35) and Kauai (N = 48) in 2009. Only five distinct haplotypes were recovered among the sequenced individuals, with haplotypes differing according to five polymorphic sites or 1.1% sequence divergence. Though haplotypes differed by 1 to 4 base pair substitutions (Figure 14), no distinct haplotype groups were recovered. As a result of the low sequence divergence observed among the cytb haplotypes, no bootstrap values greater than 50% were recovered for nodes across the reconstructed haplotype network (Figure 14).

Sequence data was also obtained for a 580 bp region of the mtDNA CO1 gene region for 125 specimens of *S. stimpsoni* sampled from four watersheds on Hawaii (N = 66) and three watersheds on Kauai (N = 59) in 2009. Six distinct haplotypes were recovered among the sequenced individuals, with haplotypes differing according to variation at ten polymorphic sites or 1.7% sequence divergence. Haplotypes differed by a maximum of nine substitutions, but did not form distinct haplotype groups. No bootstrap values greater than 50% were recovered for nodes across the reconstructed haplotype network (Figure 15). Though a more comprehensive mtDNA data collection effort was not undertaken due to the low number of haplotypes recovered in exploratory efforts, the available cytb and CO1 data were analyzed to assess genetic differentiation across the archipelago.

The prevalence and distribution of the *S. stimpsoni* cytb and CO1 haplotypes were unequal across islands. For both gene regions, two haplotypes were recovered on all islands and in all watersheds, whereas all other haplotypes were uncommon or rare. The most common cytb haplotype was recovered in 58% of sequenced individuals. The second most common cytb haplotype was recovered in 35% of sequenced individuals. A third haplotype was recovered in six individuals, with the other two cytb

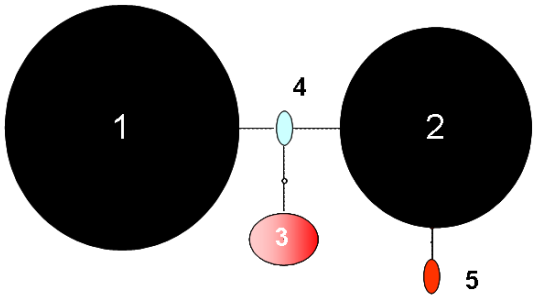

Figure 14: Cytochrome b haplotype network for *Sicyopterus stimpsoni*. Black = common to Hawaii, Maui, and Kauai; red = Kauai only; light blue = Hawaii only; red-to-pink gradient = shared between Kauai and Maui.

haplotypes found in only one individual. The two most common CO1 haplotypes were recovered in 47% and 43% of individuals, respectively. A third CO1 haplotype was recovered in nine individuals, and the other three haplotypes were recovered in one individual. The two most common cytb haplotypes were recovered on Hawaii, Maui and Kauai, and the third most common haplotype was recovered on Maui and Kauai. Both Hawaii and Kauai harbored one unique cytb haplotype. Similarly, the three most common CO1 haplotypes were recovered on both Hawaii and Kauai. Kauai also harbored two unique CO1 haplotypes and Hawaii harbored one unique CO1 haplotype.

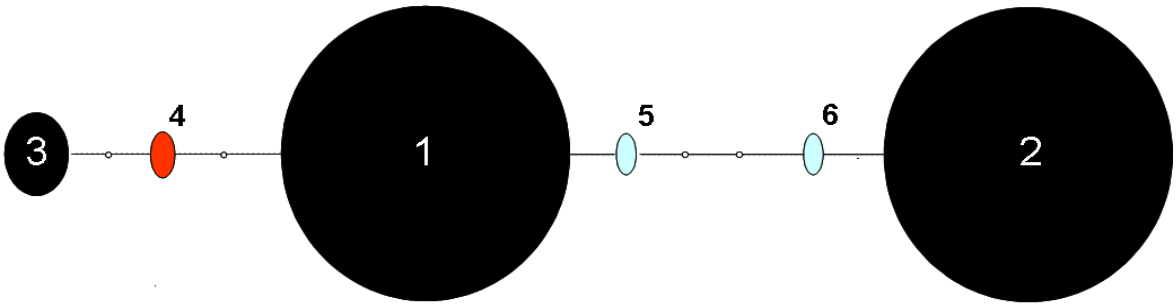

Figure 15: Cytochrome Oxidase I (CO1) haplotype network for *Sicyopterus stimpsoni*. Black = common to both Hawaii and Kauai; red = Kauai only; light blue = Hawaii only.

Though both gene regions expressed low levels of haplotype and sequence variation, the unequal distributions of haplotypes translated to significant levels of genetic differentiation among islands. For the cytb region, a significant global Φ_{ST} estimate of 0.03 ($p < 0.001$) was recovered, where approximately 3% of observed variation was attributable to haplotype frequency differences among islands. Pair-wise estimates of Φ_{ST} were -0.017 between Kauai and Maui, 0.038 between Maui and Hawaii, and 0.084 between Kauai and Hawaii. While the pair-wise comparison between Kauai and Hawaii was statistically significant ($p = 0.03$), significant differences were not found for either the pair-wise comparison between Kauai and Maui ($p = 0.67$) or the comparison between Maui and Hawaii ($p = 0.11$). Similar levels of genetic differentiation were observed for the CO1 gene region.

Discussion: As a necessary first step towards the development and use of genetic protocols for assessing oceanic island stream ecosystems, we assessed the influence of historical processes on structuring genetic variation in the native gobies *Sicyopterus stimpsoni* and *Awaous stamineus*. For each species, we characterized geographic patterns of mtDNA haplotype frequencies and sequence variation to test alternative hypotheses of evolutionary history and geographical patterns of colonization, including (1) panmixia; (2) asymmetric, directional isolation-by-distance attributable to island geomorphic development; (3) isolation-by-distance attributable to multidirectional post-colonization dispersal following an initial stochastic founding event; (4) island-specific allopatric differentiation reflective of island geomorphic development; and (5) island-specific allopatric differentiation not reflective of island geomorphic development. Better understanding of evolutionary history and historical colonization across islands can improve management of native fishes and oceanic island stream ecosystems. For example, if species exhibit island-specific evolutionary lineages, then relocation and restocking efforts should supplement at-risk populations from sources with a matching genetic profile to sustain evolutionary potential. Not doing so could result in a loss of fitness by disrupting local adaptive processes. In the absence of allopatric lineages, at-risk populations may be rescued or sustained by contributions from a wider range of potential source populations.

Several attempts have been made to determine the extent of genetic variation in native amphidromous fishes and invertebrates of the Hawaiian Islands (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998), but prior to the work presented here, a comprehensive analysis involving sufficiently informative molecular markers and exhaustive sampling had yet to be conducted. In what is arguably the most extensive prior study of genetic variation in an amphidromous species across the Hawaiian Islands, Hodges and Allendorf (1998) did not find evidence of evolutionary diversification in a survey of allozyme variation among 360 *Neritina granosa* sampled across three streams on each of four islands. However, the study did find evidence of significant genetic differentiation among islands (Hodges and Allendorf 1998). Inferences about the absence of genetic differentiation in native amphidromous fishes largely reflect two studies (Zink et al. 1996, Chubb et al. 1998) that found evidence of high mtDNA haplotype diversity within all five goby species. The results of these studies are far from conclusive, however, because data were collected from a small number of specimens. Zink et al. (1996) examined an average of 23 individuals per species (< 5 individuals per species per island), while Chubb et al. (1998) only examined an average of 14 specimens per taxon (< 3 individuals per species per island). A similarly designed study (Bebler and Foltz 2004) of all four native amphidromous invertebrates in Hawaiian streams also found evidence of high mtDNA haplotype diversity within each species, though evidence of significant population differentiation was recovered for two of the species (*Atyoida bisulcata*, *Neritina vespertina*) between Kauai and Hawaii.

A rich literature on terrestrial, semi-aquatic, and anchialine biota in Hawaii indicates that more comprehensive approaches can reveal within-species evolutionary history and historical biogeography. The distribution of mtDNA lineages, mtDNA haplotype frequencies and multi-locus genotype frequencies have served as evidence of allopatric speciation and evolutionary diversification in terrestrial arthropods, vertebrates and plants (e.g., Funk and Wagner 1995, Fleischer et al. 1998, Roderick and Gillespie 1998, Lovette et al. 2002, Givnish et al. 2009). Similar evidence has served to identify other factors, including sexual selection, that have

spurred evolutionary diversification of terrestrial fauna across the archipelago (e.g., Mendelson and Shaw 2005). Studies also suggest that allopatric isolation has figured prominently in the evolutionary diversification of semi-aquatic and anchialine fauna. For example, through phylogenetic reconstruction, Jordan et al. (2003) found that the diversity of semi-aquatic *Megalagrion* damselflies is attributable to allopatric isolation following stepping-stone colonization of islands emerging from the sea, with molecular clock estimates suggesting that the progenitor lineage(s) of *Megalagrion* arrived in the archipelago about 10 million years ago, well before the emergence of Kauai. The distribution of evolutionary lineages also suggests that subsequent diversification on islands appears linked to ecological specialization (Jordan et al. 2003) and isolation arising from sea level fluctuation (Jordan et al. 2005). Finding evidence of extensive geographical structure of mtDNA haplotype frequencies in anchialine *Halocaridina* shrimp, Santos (2006) and Craft et al. (2008) inferred that a combination of intrinsic organismal attributes (e.g., restricted larval habitat) and extrinsic barriers to dispersal (e.g., oceanic barriers and geological compartmentalization of island aquifers) have given rise to micro-geographically distributed evolutionary lineages across the island of Hawaii following two distinct colonization events.

Because amphidromy can involve marine larval dispersal (see section 3.0), the evolutionary history of native stream fishes in Hawaii may be more like broadcast-spawning littoral marine fauna than terrestrial, semi-aquatic or anchialine biota. Marine environments present few impermeable physical barriers to larval dispersal. Accordingly, little evidence of within-archipelago evolutionary differentiation has been found among marine fauna (Kay and Palumbi 1987, Cunha et al. 2005), including most broadcast-spawners in the Hawaiian Islands (Meyer et al. 2005, Bird et al. 2011). Bird et al. (2011), however, found that the evolutionary history of Hawaiian *Cellana* limpets is attributable to a rare colonization event 3.4-7.2 MYA from the vicinity of Japan, followed by diversification driven by natural selection along an ecological gradient within the littoral zone of islands across the archipelago. Hawaiian *Cellana* limpets also exhibit significant population structure and isolation by distance, indicative of restricted gene flow across channels separating islands (Bird et al. 2007). The distribution of genetic variation within *Cellana* species does not follow the progression rule (i.e., where older lineages or greater genetic diversity occurs on older islands), which suggests that colonization and subsequent spread did not track island geomorphic development (Funk and Wagner 1995). Rather, evidence of a more complex history was recovered. For example, mtDNA CO1 haplotypes exhibited by *C. talcosa* on Kauai are not found on the other main Hawaiian Islands (Bird et al. 2007). Haplotype diversity in *C. talcosa* and *C. exarata* also was found to decline along a southeast to northwest axis (Bird et al. 2007), suggesting that colonization progressed from younger to older islands. There are many ecological and life history differences between amphidromous and littoral zone fauna (e.g., larval life duration), but these findings nonetheless indicate that evolutionary differentiation of species with marine dispersing larvae may proceed without complete geographic segregation within the Hawaiian archipelago.

Though the evolutionary history and historical biogeography of native amphidromous fauna in the Hawaiian Islands are not well studied, it has been proposed that each species originated through independent colonization, and that panmictic spread throughout the archipelago has sufficiently dampened genetic differentiation to prevent subsequent evolutionary diversification (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998, Hodges and Allendorf 1998,

McDowall 2003, Bebler and Foltz 2004). The presence of goby species from five different genera encompassing two different families is strong evidence for independent colonization of the Hawaiian Islands (McDowall 2003, 2010). However, the hypothesis of post-colonization spread throughout the archipelago has not been adequately tested. Prior studies have largely involved limited sampling (e.g., Zink et al. 1996, Bebler and Foltz 2004) and often did not present information on geographical patterns of genetic variation through the lens of evolutionary history and historical biogeography. For example, the hypothesized absence of island-specific monophyletic lineages in native gobies has been interpreted as evidence of regional source-sink dynamics driven by larval exchange among islands (Zink et al. 1996, Chubb et al. 1998). Prior studies have also discounted the value of more comprehensive assessments of genetic variation, suggesting that further sampling would not likely uncover patterns that would challenge the prevailing idea of panmixia across the archipelago (Bebler and Foltz 2004).

Here we show that at least one species of native amphidromous fish exhibits patterns of genetic differentiation among islands that, like littoral zone limpets, do not follow the progression rule (Funk and Wagner 1995, Bird et al. 2007). The diversity and distribution of mtDNA haplotypes in *Awaous stamineus*, and to a lesser extent *Sicyopterus stimpsoni*, suggest that the historical biogeography of native amphidromous fauna in the Hawaiian Islands is more complex, and that it may only secondarily reflect island geomorphic development. A combination of among-archipelago and within-archipelago analyses provides evidence of isolation-by-distance attributable to multidirectional, post-colonization spread following an initial stochastic founding event. Among-archipelago phylogeographic comparisons (Lindstrom et al. 2012) indicate it is unlikely that arrival to the archipelago predated the formation of the main Hawaiian Islands, as has been found in some terrestrial species (Price and Clague 2002) that appear to have originated or diversified on land masses that are now submerged. Rather, arrival most likely occurred well after the formation of Kauai via long-distance dispersal from a source in the western Pacific (Lindstrom et al. 2012). Within-archipelago phylogeographic comparisons provide evidence of demographic expansion and multi-directional spread among islands following arrival. Signatures of significant genetic heterogeneity and population structure also serve as evidence of restricted dispersal across the archipelago.

The arrival of *Awaous* to the Hawaiian archipelago can be inferred from genetic comparison of populations in Hawaii and Guam. Lindstrom et al. (2012) recovered a minimum of 1.7% sequence divergence across the mtDNA cytb region for *Awaous* sampled from Hawaii and Guam. Working under the assumption that *A. guamensis* in the Mariana Islands is the progenitor of *Awaous* in Hawaii, application of the observed mutation rate at the cytb gene region for other Gobiids of 1.93-2.17% sequence divergence per million years (Rocha et al. 2005) suggests that *Awaous* colonized the Hawaiian archipelago approximately one million years ago (Lindstrom et al. 2012). Thus initial colonization likely was not restricted to Kauai or an older island that has since become submerged. Rather, colonization more likely occurred on Kauai, Oahu, or the western region of the Molokai-Maui complex referred to as Maui Nui, which all arose between 1.32 to 5.1 MYA. The hypothesized timing of the arrival of *Awaous* to the Hawaiian Islands is consistent with evidence suggesting that a diverse range of terrestrial and semi-aquatic fauna colonized the archipelago following the formation of Kauai (Price and Clague 2002). For example, molecular estimates suggest that native Hawaiian waterbirds, such as the Hawaiian

duck (Anas *wyvilliana*) and Hawaiian stilt (*Himantous mexicanus knudsenii*), both colonized the archipelago approximately 0.75-1.5 MYA (Fleischer and McIntosh 2001).

The distribution of mtDNA sequence variation and haplotype frequencies indicate that *Awaous* initially colonized Oahu and subsequently dispersed to other islands in the archipelago. Populations of *A. stamineus* on Oahu harbor the greatest number of unique haplotypes and shared haplotypes, which suggests that *Awaous* colonized Oahu upon arriving to the archipelago. Haplotype diversity can sometimes be driven by contemporary demography, where higher diversity corresponds to greater population size. Evidence of depressed population densities of native fishes across Oahu (see section 5.1.2) indicates that the observed pattern of unique and shared haplotype diversity on Oahu is not attributable to contemporary demography. Mismatch distribution tests recovered a signature of demographic expansion across the entire archipelago, and tests restricted to individual islands recovered an expansion signature only on Oahu, suggesting that post-colonization spread originated there. A combination of evidence, including comparably high numbers of unique and shared haplotypes as well as the highest observed values of haplotype and nucleotide diversity, suggests that *A. stamineus* dispersed to eastern Maui Nui (i.e., Maui) from Oahu. Relative values of unique haplotypes as well as haplotype and nucleotide diversity indicate that the range of *A. stamineus* then expanded to Hawaii, Molokai and Kauai. Estimates of population differentiation based on mtDNA sequence variation indicate that following founding events, exchange among islands has been limited, particularly among Hawaii and all other islands. Significant genetic differentiation also was found among neighboring islands and islands that were connected during glacial maxima (i.e., Molokai and Maui). These patterns, along with a weak trend of isolation-by-distance, suggest that range expansion proceeded from proximate sources rather than aggregate contributions via long-distance dispersal.

Less information is available on the historical biogeography and evolutionary history of *Sicyopterus stimpsoni*, but some of the observed patterns of genetic variation parallel those observed in *A. stamineus*. A truncated analysis recovered comparably lower levels of mtDNA sequence variation and haplotype diversity than *A. stamineus*, suggesting that *S. stimpsoni* is of equivalent or younger age. This is consistent with phylogenetic evidence indicating that the age of other Indo-Pacific *Sicyopterus* lineages correspond to divergence events that occurred between 1 and 6 million years ago (Keith et al. 2005). Thus, it is likely that *Sicyopterus* colonized the Hawaiian archipelago after the formation of Kauai, with patterns of global phylogeography in Sicydiinae gobies suggesting that the source was located in the western Pacific (Keith et al. 2011). Though the available information does not provide a basis for inferring the progression of post-colonization spread, evidence of genetic differentiation between Kauai and Hawaii suggests that range expansion likely proceeded from proximate rather than distant sources, and that exchange among islands has been limited following founding events. Further analysis, perhaps relying on data from additional mtDNA gene regions, might clarify whether *S. stimpsoni* and *A. stamineus* exhibit common or incongruent biogeographic histories (Taillebois et al. 2013).

The inferred origins and spread of *A. stamineus* and *S. stimpsoni* in the Hawaiian Islands are similar to the historical biogeography of diadromous species elsewhere in the Indo-Pacific and Caribbean basin. For example, the distribution of mtDNA sequence variation and haplotype

groups in *Sicyopterus lagocephalus*, a recently derived but widespread congener in the Indo-Pacific, suggests that genetic divergence followed an initial phase of peripheral colonization from an ancestral population, and that a secondary phase of centrifugal colonization resulted in further range expansion (Hoareau et al. 2012). The timing of demographic and range expansion (6.6-9.9 KYA and 98.1-174.6 KYA) appears to have tracked changes in sea level corresponding to the two most recent Pleistocene glacial-interglacial climatic cycles (Hoareau et al. 2012). This suggests that the expansion and spread of amphidromous species across the Hawaiian Islands might also have corresponded to fluctuations in sea level as well as the progression of island formation. Similar findings in anadromous lineages of *Leucopsarion petersii* across the Japanese archipelago (Kokita and Nohara 2010), and eastern Pacific populations of *Sicydium salvini* (Chabarria and Pezold 2013) provides further support for this possibility. Reconstructions of the colonization history of Puerto Rico also illustrate that the assembly of oceanic island stream communities can be a multi-phase process (Cook et al. 2008, 2010). Estimates of population expansion across 11 amphidromous species, including 3 species of *Sicydium* gobies, provide evidence of a continuous colonization history spanning the late Pleistocene and Holocene (~2-60 KYA) that can be partitioned into three phases that differ in the average rate of species arrival to the island (Cook et al. 2010). Though further comparisons involving more extensive data for *S. stimpsoni* are warranted, this supports our inference that *Awaous* and *Sicyopterus* likely share a common historical biogeography in the Hawaiian Islands. Further comparisons involving other amphidromous species would also serve to test this hypothesis, and provide a broader context for relating contemporary demography (see sections 5.0.1-5.0.2) to the historical demography of at-risk species across the archipelago.

5.0.2 Genetic Analysis of Contemporary Connectivity

Population genetic structure in Awaous: Microsatellite-based estimates of population differentiation (F_{ST}) among all island and watershed populations were calculated for *Awaous stamineus* in 2009 and 2011. In both years, population differentiation was very low. In 2009, pairwise F_{ST} values ranged from -0.001 to 0.001 among island populations with a global F_{ST} of -0.0002. At the watershed scale, F_{ST} values ranged between -0.16 to 0.14 in 2009 with a global F_{ST} of 0.005. The greater range in F_{ST} values at the watershed level likely reflects greater variation in sample sizes, as samples sizes were more similar among islands. None of the pairwise comparisons between islands or watersheds were significantly different from the null hypothesis of no differentiation between populations in 2009 (i.e., $F_{ST} = 0$). In 2011, F_{ST} values ranged from -0.0005 to 0.007 among island populations with a global F_{ST} of 0.004. At the watershed scale, F_{ST} ranged between -0.02 to 0.08 with a global F_{ST} of 0.005. At the island scale, pairwise comparisons between Oahu and all other islands were significant ($p = 0.001$) indicating weak differentiation. Watershed-scale comparisons did not result in significant differences, possibly as a result of smaller effect sizes from smaller sample sizes. Correction of F_{ST} estimates for the number of alleles at a locus (Jost 2008) did not improve the detection of differentiation. Jost's D_{est} values were as low as F_{ST} estimates. In 2011, for example, values ranged between 0.00005 and 0.005.

***Bayesian population assignment in Awaous*:** Iterations of the Bayesian assignment algorithm in STRUCTURE were run to detect patterns of population differentiation in *A. stamineus* across the

archipelago. Analyses assumed conditions of admixture among putative populations (if present) and island population identity was used as prior information to improve the likelihood of detecting very subtle patterns of differentiation. In 2009, Bayesian estimates of population structure found little evidence of differentiation among islands (Figure 16) which is consistent with F_{ST} results. However, in 2011, Bayesian approaches found evidence of two divergent populations (Figure 16) that were not detected by more coarse statistics like F_{ST}. In 2011, there appears to be a western population and an eastern population (Figure 16). Individuals collected on the island of Hawaii are the archetype of the western population and show very little admixture (i.e., gene flow) with the eastern population. Moving eastward among islands to Maui and Molokai, there is an increasing level of gene flow between the western and eastern populations although the individuals are still largely assigned to the western population, indicating low levels of gene flow from the eastern population to locations on Maui and Molokai. There is a greater amount of admixture between the western and eastern populations on Oahu. There are also individual fishes that were assigned primarily to the East population, indicating recent dispersal from Kauai (i.e., within one generation time). On the island of Kauai, most residents were assigned to the eastern population. There are a few individuals that were assigned to the western population and some individuals that are clearly hybrids between the two indicating low levels of dispersal from the western archipelago to Kauai and interbreeding between the eastern and western populations. This pattern is consistent with high levels of dispersal from west to east, with a moderate dispersal barrier between Oahu and Kauai.

The limited presence of individuals assigned to the eastern population on Oahu and admixed individuals on Molokai and Maui suggest that dispersal from east to west is much less likely than west to east dispersal. It also suggests that east-to-west dispersal may happen in a stepping-stone manner over the span of several generations. The lack of admixture between eastern and western populations on the island of Hawaii indicates that there may also be a barrier to west-to-east dispersal between Maui and Hawaii. These results are consistent with the results of biophysical modeling simulations (see section 5.0.5) as well as with previous studies of genetic breaks in the Hawaiian archipelago (Toonen et al. 2011).

Assignment of Awaous postlarvae to source populations: To determine the degree of contemporary larval dispersal among island populations, newly arrived postlarvae were assigned to putative adult source populations based on their multi-locus genotype probabilities using a stringent two-step method. First, a Bayesian assignment method was used (Rannala and Mountain 1997), with probability estimates generated by 10,000 iterations of Monte Carlo re-sampling in GENECLASS v2.0. Second, a rank-based assignment method was applied where individuals were assigned based on their negative log-likelihood of belonging to one population relative to the other source populations (Rannala and Mountain 1997). Individuals were considered assigned when they met two criteria:

1) they must be assigned to at least one source population with a probability greater than 85% in the first Monte-Carlo based assignment procedure. Those with probabilities < 85% were considered unassignable.

2) the individuals that met the 85% threshold in the first step were then assessed using the rank-based method. Individuals were assigned to the source population if the likelihood of assignment

to the highest ranked source population was 2-times greater than the likelihood of assignment to the next highest ranked population.

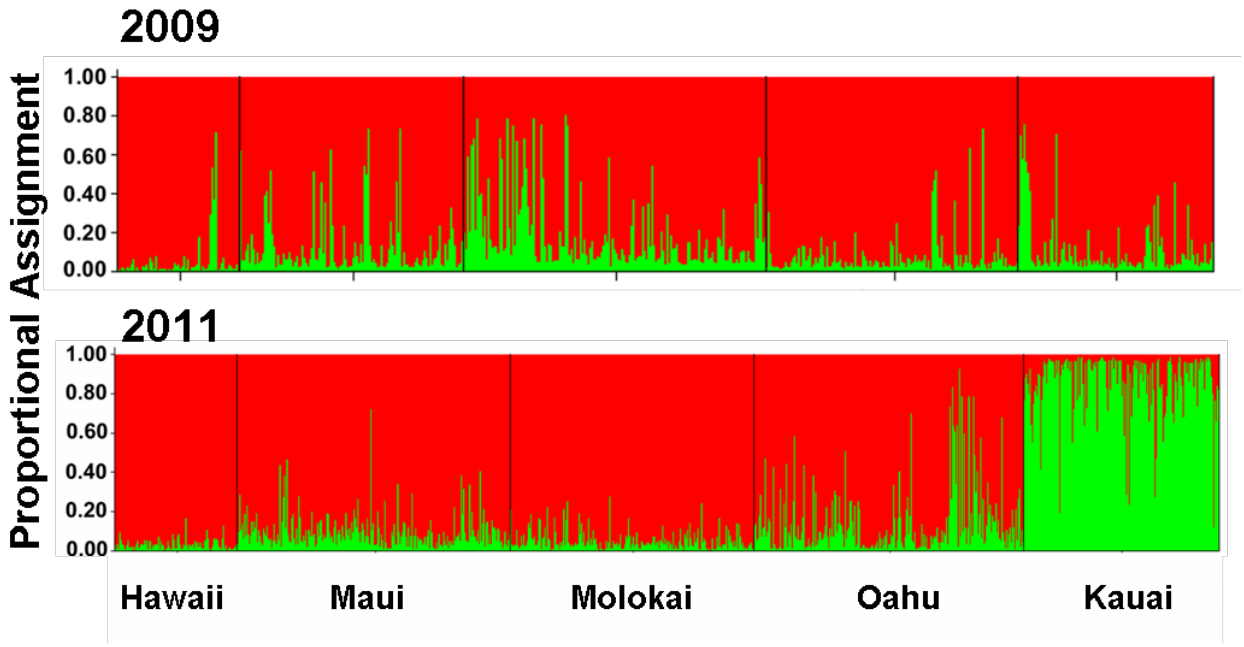

Figure 16: Bayesian population assignment results for *Awaous stamineus* populations across the Hawaiian achipelago in 2009 (top) and 2011 (bottom). The most likely number of populations of *A. stamineus* in 2009 was 1. The most likely number of populations of *A. stamineus* in 2011 was 2: an eastern population (in red) and a western population (in green). Bar graphs are representative of one iteration of K = 2 with 500,000 iterations and 250,000 burn-in.

Evidence of long distance dispersal and local retention was recovered in source-destination comparisons restricted to the 2009 data (Figure 17). For example, source populations on all islands contributing postlarvae to streams on both Kauai and Hawaii, and estimates indicate that 67% of all postlarvae produced in streams on Hawaii recruited to the island of Kauai. The proportion of locally sourced postlarvae was low on some islands (e.g., 0% for Hawaii and Molokai), but it ranged as high as 37% on Kauai. There was variation among island populations in the production of postlarvae. Hawaii and Molokai produced the fewest assignable postlarvae (n = 6 each), whereas Maui and Oahu produced the most assignable postlarvae (17 and 18 respectively).

In 2011, larval dispersal appeared to match the results from Bayesian analyses (Figure 17), indicating reduced dispersal between locations on western islands and Kauai. Furthermore, postlarvae produced in streams on Kauai were most likely to recruit to Kauai (5%) and its nearest neighbors to the west, Oahu (90%) and Molokai (5%). This pattern also parallels results from Bayesian analyses of population connectivity. As in 2009, local retention varied among islands in 2011 from 0% (Maui and Molokai) to 50% (Hawaii). Maui produced the fewest assignable postlarvae in 2011 (n = 4) whereas Kauai produced the most (n = 19). These patterns indicate that the return of locally produced larvae is a common and frequent event for *A. stamineus* across the species entire range, though the apparent differences in connectivity matrices from 2009 and 2011 indicate that there is considerable variation in larval dispersal over time.

***Population genetic structure in Sicyopterus stimpsoni*:** Estimates of population differentiation (F_{ST}) were calculated among all island and watershed populations for *Sicyopterus stimpsoni* in both 2009 and 2011. In both years, population differentiation was very low. In 2009, pairwise F_{ST} values ranged from -0.003 to 0.001 among island populations with a global F_{ST} of 0.001. At the watershed scale, F_{ST} ranged between -0.33 to 0.27 in 2009 with a global F_{ST} of -0.002. As with *A. stamineus*, the greater range in F_{ST} values at the watershed level likely reflect greater variation in sample sizes. None of the pairwise comparisons between islands or watersheds were significantly different from the null hypothesis of no differentiation between populations in 2009 (i.e., $F_{ST} = 0$). In 2011, F_{ST} values ranged from -0.0003 to 0.001 among island populations with a global F_{ST} of 0.0003. At the watershed scale, F_{ST} ranged between -0.03 to 0.08 with a global F_{ST} of 0.001. No pairwise comparisons showed significant differences from the null hypothesis of no differentiation.

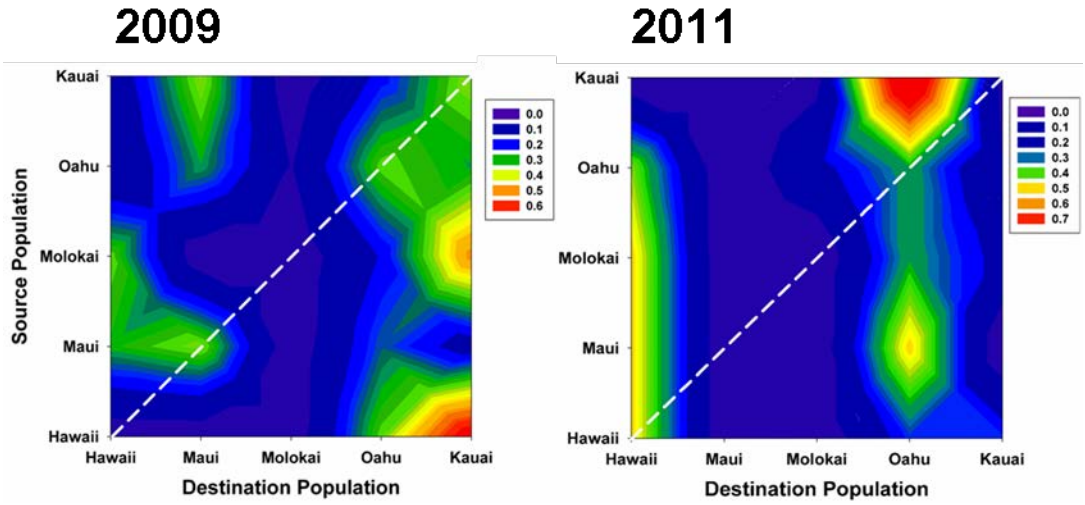

Figure 17: Genetic contours plots of larval dispersal between source and destination island populations of *Awaous stamineus* in 2009 (left) and 2011 (right); contours indicate proportions of postlarvae assigned to a particular source site (rows) that dispersed to the various island destinations (columns). The proportion assignments in each row add to 1. The diagonal dashed line indicates the proportion of locally produced postlarvae in a particular population.

***Bayesian population assignment in Sicyopterus stimpsoni*:** Iterations of the Bayesian assignment algorithm in STRUCTURE were run to detect patterns of population differentiation in *Sicyopterus stimpsoni* across the archipelago. Analyses assumed conditions of admixture among putative populations (if present) and island population identity was used as prior information to improve the likelihood of detecting very subtle patterns of differentiation. In both 2009 and 2011, Bayesian analysis of population structure found little to no differentiation among islands or watersheds (Figure 18), where the likeliest scenario was a single population across the archipelago and widespread gene flow.

***Assignment of Sicyopterus postlarvae to source populations*:** To determine the degree of contemporary larval dispersal among island populations, newly arrived postlarvae were assigned to putative adult source populations based on their multi-locus genotype probabilities using the two-step method described above. Evidence of long distance dispersal and local retention was recovered in source-destination comparisons restricted to the 2009 data. For example, source populations on all islands (with the exception of Oahu) contributed postlarvae to streams on both

Kauai and Hawaii (Figure 19). Assignment estimates suggest that 90% of all postlarvae produced in streams on Maui recruited to the island of Kauai, and 60% of postlarvae produced on Kauai recruited to Hawaii. All islands (with the exception of Oahu) contributed postlarvae recruits to Kauai and Kauai contributed recruits to all other islands (Figure 19). The proportion of locally sourced postlarvae was much less variable than *A. stamineus*, ranging between 0% (Hawaii and Molokai) to 20% (Kauai). The largest producers of assignable postlarvae were Maui and Molokai (10 and 13 respectively) whereas no postlarvae were assigned to Oahu. In 2011, gross patterns of larval dispersal matched that of 2009, providing further evidence of long distance dispersal. However, local-retention of larvae varied widely among islands in 2011 from 0% (Oahu) to 40% (Kauai), which indicates that the return of locally produced larvae is a common, frequent event for *S. stimpsoni* across the species entire range. Similar to 2009, the largest producers of postlarvae were Maui (n = 39) and Molokai (n = 76) that seeded populations on all

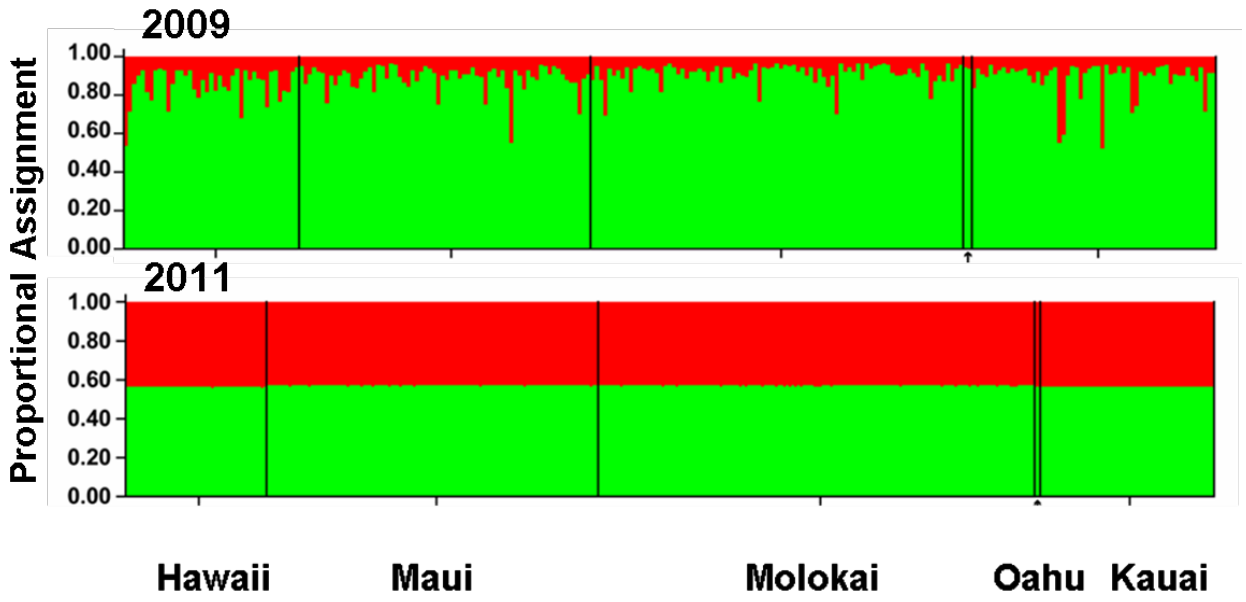

Figure 18: Bayesian population assingment results for *Sicyopterus stimpsoni* populations across the Hawaiian achipelago in 2009 (top) and 2011 (bottom). The most likely number of populations of *S. stimpsoni* in 2009 and 2011 was 1. Bar graphs are representative of one iteration of K = 2 with 500,000 iterations and 250,000 burn-in.

islands (except Oahu). Again, Oahu produced no assignable postlarvae in 2011, indicating that Oahu is a larval sink that does not meaningfully contribute to the metapopulation. Similar to *A. stamineus*, there appears to be some temporal variation in larval dispersal when the connectivity matrices were compared between 2009 and 2011 at the site-pair level. For example, Hawaii supplied a relatively large proportion of postlarvae to Oahu in 2011 (67%), but much less by proportion in 2009 (36%).

Discussion: As a necessary step towards the development and use of genetic protocols for assessing oceanic island stream ecosystems, we re-assessed population genetic structure and contemporary connectivity of *Sicyopterus stimpsoni* and *Awaous stamineus* across the Hawaiian Islands. For each species, we characterized nuclear microsatellite allelic differentiation to assess geographic patterns of genetic variation in order to test the hypothesis that recruitment draws

from mixed immigrant pools due to larval exchange among islands. This involved estimating the extent of genetic differentiation among islands and determining the likeliest source population of postlarvae captured during stream re-entry.

Though several attempts have been made to characterize archipelago-wide patterns of genetic variation within each of the gobies native to the Hawaiian Islands (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998), prior to the work presented here, a comprehensive analysis involving sufficiently informative molecular markers and exhaustive sampling had yet to be conducted. Moreover, no study had directly assessed movement potential and contemporary population connectivity. Prior studies of native Hawaiian stream fauna have largely involved limited sampling (e.g., Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998, Bebler and Foltz 2004) and often interpreted patterns reflecting historical biogeography as evidence of contemporary population connectivity and dispersal potential (e.g., Zink et al. 1996, Chubb et al. 1998, Bebler and Foltz 2004). For example, the absence of island-specific monophyletic lineages in native gobies has largely been interpreted as evidence of source-sink dynamics driven by larval exchange among islands instead of evidence of post-colonization demographic expansion

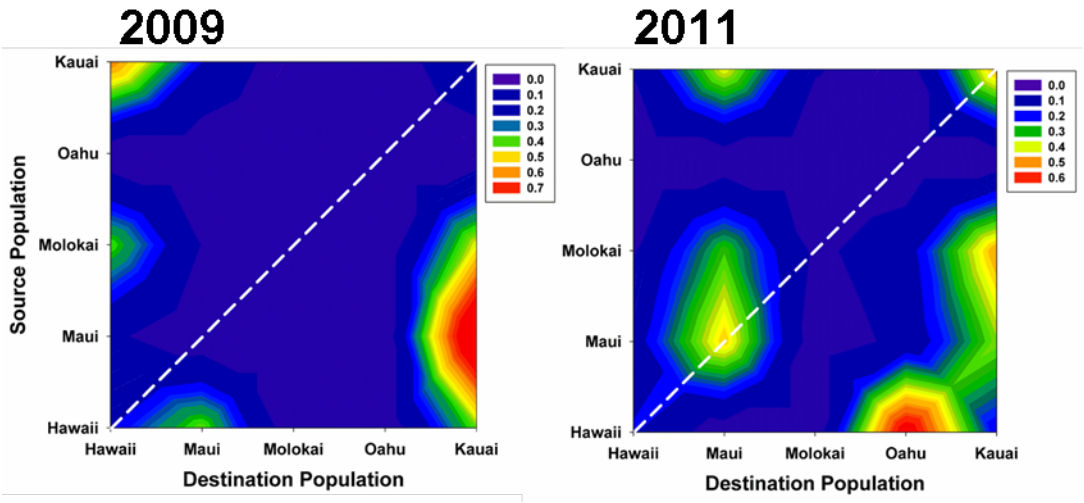

Figure 19: Genetic contour plots of larval dispersal between source and destination island populations of *S. stimpsoni* in 2009 (left) and 2011 (right); contours indicate proportions of postlarvae assigned to a particular source site (rows) that dispersed to the various island destinations (columns). The proportion assignments in each row add to 1. The diagonal dashed line indicates the proportion of locally produced postlarvae in a particular population.

and spread following arrival to the archipelago (see section 5.0.1). Though far from conclusive, prior studies nonetheless promulgated the hypothesis that contemporary gene flow is high in native amphidromous fishes and invertebrates (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998, Hodges and Allendorf 1998, Bebler and Foltz 2004), where local populations recruit from mixed immigrant pools and that population dynamics are regional rather than local due to larval exchange among islands.

Geographic patterns of contemporary connectivity did not always track patterns of genetic variation attributable to historical biogeography. Though dispersal often defines both historical and contemporary conditions, the physical and biotic processes that drive dispersal can diverge

over time. Similarly, the relative contributions of ecological and evolutionary processes can give rise to incongruent signatures of genetic variation that can sometimes only be captured through comparative approaches. Examining multiple genetic data sets can identify subtle but defining signatures of historical biogeography and reveal highly informative incongruencies that might not otherwise come to light. Comparison of mtDNA sequence variation to the microsatellite allelic variation indicates, for example, that historical patterns of colonization contrast with contemporary dispersal of *A. stamineus* across the Hawaiian archipelago. The distribution of mtDNA sequence variation reflects a sequence of initial colonization, post-colonization demographic expansion, and stepwise spread of *A. stamineus* in different directions across the archipelago. Patterns of mtDNA sequence and haplotype diversity suggest that populations on Oahu acted as a source for colonization of Maui, and that both Oahu and Maui served as sources for other areas of the archipelago across evolutionary time (see section 5.0.1). Significant differentiation among islands also suggests that post-colonization exchange among islands has been limited, particularly between Hawaii and other islands. Frequentist and Bayesian analyses of *A. stamineus* microsatellite allelic variation, on the other hand, suggest that connectivity is constrained across an east-to-west axis and contemporary dispersal is limited between Kauai and other islands in the archipelago. Not only does this comparison illustrate the pitfalls of interpreting patterns reflecting historical biogeography as evidence of contemporary population connectivity, it also provides a more expansive perspective on dispersal than those offered from prior studies that have tended to focus on consistent patterns of high genetic diversity and weak genetic differentiation.

Studies of other island taxa provide corroborative support for the inferences we have drawn from patterns of genetic variation observed in *A. stamineus* and *S. stimpsoni*. Though a number of recent studies have found evidence of limited genetic differentiation in amphidromous species across the Caribbean basin and areas of the Indo-Pacific (e.g., Cook et al. 2009, Crandall et al. 2009, Cook et al. 2012, Page et al. 2012, Castelin et al. 2013), a comparative study of genetic connectivity provides evidence of restricted gene flow and multiple barriers to dispersal across the Hawaiian Islands within and among 27 species capable of marine dispersal (Toonen et al. 2011). The majority of the species included in the study exhibit concordant patterns of genetic differences between Hawaii and Kauai and regions occurring to the northwest of Kauai. Comparisons identified the presence of common barriers to marine dispersal between Hawaii and Maui as well as between Oahu and Kauai. A large proportion of species also exhibit genetic differentiation between Oahu and the Maui Nui complex (Toonen et al. 2011). This suggests that the archipelago can be divided in to a minimum of five distinct ecoregions with limited exchange. The prevalence of exchange within and among the hypothesized ecoregions suggests that distance alone is a poor predictor of dispersal limitations. The distance between ecoregions varies considerably- from 45km between Hawaii and Maui to upwards of 180 km between the Gardner Pinnacles and French Frigate Shoals in the region of the archipelago northwest of Kauai- and few species exhibit genetic evidence of isolation-by-distance (Toonen et al. 2011). Given the diversity of species that exhibit congruent patterns of genetic differentiation, it is nonetheless likely that dispersal potential and connectivity are dictated by abiotic factors such as dominant marine currents and circulation patterns that establish 'oceanographic distances' among locations in the archipelago (Toonen et al. 2011).

Here we show that both *A. stamineus* and *S. stimpsoni* exhibit very low levels of contemporary population genetic differentiation, although comparisons indicate that, unlike *S. stimpsoni*, *A.*

stamineus exhibits significant genetic differentiation across the Hawaiian archipelago. Bayesian analysis of genetic structure detected evidence of weakly differentiated eastern and western populations in *A. stamineus*. As in many other species with marine larval dispersal (Toonen et al. 2011), *A. stamineus* exhibits restricted gene flow across the channel separating Oahu and Kauai. Dispersal also appears to be asymmetric among islands, with the east-to-west bias being consistent with the prevailing direction of ocean currents (Figure 7). No evidence of genetic differentiation was found for *S. stimpsoni*, either corresponding to oceanic circulation or biogeographic barriers. Nonetheless, patterns of contemporary connectivity indicate that *S. stimpsoni* is not panmictic across the archipelago.

Contrary to the prevailing hypothesis of range-wide panmixia, estimates of movement potential and population connectivity based on assignment tests suggest that local retention is common in both species. Though *A. stamineus* and *S. stimpsoni* can exhibit a lengthy oceanic larval dispersal phase, their maximum movement potential may not always be realized (see section 5.0.3). Genetic evidence of local retention and dispersal asymmetries suggests that marine dispersal is neither obligatory nor is it random in either *A. stamineus* or *S. stimpsoni*. The balance of local retention and dispersal varies among islands and by species- movement potential appears to be more limited in *A. stamineus* than *S. stimpsoni*, for example, and the composition of *S. stimpsoni* immigrant pools appears to be more asymmetric than the pool of *A. stamineus* immigrants- possibly as a consequence of life history variation within and among species as well as variation in population density among islands.

The observed discrepancies in genetic differentiation and contemporary connectivity between *A. stamineus* and *S. stimpsoni* are consistent with unanticipated evidence of life history variation in *A. stamineus*. Estimates of larval life duration suggest that *A. stamineus* would exhibit lower genetic differentiation and greater connectivity than *S. stimpsoni*. Both species can exhibit a marine larval dispersal phase, and marine-dispersing *A. stamineus* exhibit a longer average larval life duration in (~160 days, Radtke et al. 1988) than the range of larval life durations observed in endemic Sicydiine gobies (~53-106 days, Radtke et al. 2001, Lord et al. 2010, Taillebois et al. 2012,), including *S. stimpsoni* (~101 days, Hogan et al. unpublished). Evidence to the contrary suggests that genetic differentiation and connectivity do not necessarily scale to larval life duration. The balance of local retention versus marine larval dispersal could be an equally or more important determinant. Newly available data on otolith microchemistry indicates that *A. stamineus* is facultatively amphidromous (see section 5.0.3), where the majority of larvae of *A. stamineus* do not enter marine environments, and where many of the larvae that do enter the ocean remain close to shore. This is consistent with recent studies showing that local retention and natal homing of pelagic larvae are stronger among marine dispersing fishes than has been previously thought (Thorrold et al. 2001, Taylor and Hellberg 2003, Pampoulie et al. 2004, Sorenson et al. 2005). By increasing the likelihood of natal homing and return to nearby watersheds on the same island, facultative amphidromy can result in stronger patterns of genetic differentiation and lower connectivity among islands than what would be predicted based on estimates of larval life duration. However, as comparisons between 2009 and 2011 demonstrate, the extent of differentiation and connectivity can be dynamic because shifts in local conditions may differentially promote or discourage marine larval dispersal (i.e., a decline in local conditions may result in more larvae entering the ocean). Indeed, evidence of greater genetic differentiation in 2011 parallels otolith microchemistry results indicating that a greater

proportion of the sampled individuals (~80% versus ~60%) did not undergo marine dispersal compared to individuals sampled in 2009 (i.e., with an average lifespan of roughly 2 years, most individuals captured in 2011 were born during or after 2009). Though little is known about life history variation in *S. stimpsoni*, some of our findings suggest that the species either is not obligately amphidromous, or that a significant proportion of larvae remain in nearshore marine environments. For example, the maximum proportions of locally sourced postlarvae were similar for *A. stamineus* and *S. stimpsoni* (50% and 40%, respectively). Subjecting this hypothesis to greater scrutiny could show whether the observed discrepancies in genetic differentiation and connectivity between *A. stamineus* and *S. stimpsoni* reflect categorical differences (i.e., facultative versus obligatory amphidromy) or incremental differences in life history or larval ecology (i.e., longer versus shorter larval life duration or differential use of marine habitat by larvae). Expanding the scope of additional comparisons to other species would be useful for understanding the role of life history variation in structuring connectivity and genetic variation across all native Hawaiian gobies.

The observed differences in contemporary connectivity between *A. stamineus* and *S. stimpsoni* also are likely a consequence of variation in population densities and dispersal asymmetries across the archipelago. With a few important exceptions, source-tracking of recruiting postlarvae indicated that local populations recruit a mixed pool of immigrants originating from the same island and other islands in the Hawaiian archipelago, where the degree of admixture varies among islands and over time. The composition of immigrant pools likely corresponds to differences in larval productivity among local populations (Bell 1994, Bell 1999, Chong 2000, Luton et al. 2005, Bell 2007), though life history variation may serve to counterbalance or compensate for variation in larval productivity. If marine dispersal is favored under impaired conditions, then declining populations might contribute a disproportionate quantity of larvae to immigrant pools even when larval production is comparably low (Luton et al. 2005). However, under conditions of severe impairment, larval productivity may be so low that at-risk populations simply do not contribute to the immigrant pool. Large imbalances in larval productivity could result in source-sink dynamics among watersheds or among islands. Some of our results conform to this hypothesized scenario. For example, the proportions of assignable *A. stamineus* postlarvae were relatively even among islands, despite widespread impairment and depressed population densities on Oahu (see section 5.1.2). Our results also indicate that Oahu is a demographic sink for *S. stimpsoni*; the species is rare on the island (Hawaii Watershed Atlas; http://hawaii.gov/dlnr/dar), and postlarvae recruit to Oahu from other islands but no postlarvae arriving on other islands appear to have been produced on Oahu.

Asymmetries in the composition of immigrant pools and connectivity across the archipelago may also be attributable to the location of source populations relative to prevailing ocean circulation. In the Hawaiian archipelago, where directional currents move east to west across the windward face of the islands (i.e., where the majority of perennial streams are located), immigrant pools might be dominated by local sources and sources from eastern populations. Deviations from this expectation may occur as a result of geographic barriers to dispersal (Toonen et al. 2011), the influence of other oceanic currents, and variation in population densities within and among islands that promote greater productivity and retention of larvae in off-shore currents. If the composition of immigrant pools is dictated solely by directional currents, then (1) easterly biases in admixture would be likely to occur, (2) few, if any immigrants from Kauai would recruit to

other islands; and (3) immigrants recruiting to Kauai would be more admixed than immigrants to Hawaii. The influence of other oceanic currents (e.g., gyres) would likely increase the proportional contributions of centrally located islands, and variation in population densities would likely decrease the proportional contributions of populations on Oahu and increase the proportional contributions of populations on Molokai (see text below). Source-tracking of *A. stamineus* and *S. stimpsoni* postlarvae indicate that admixture is not driven solely by directional currents. Other factors, such as life history variation, appear to be influencing the composition of *A. stamineus* immigrant pools. Genetic estimates indicate that populations on Maui and Molokai are the primary sources of *S. stimpsoni* postlarvae that recruit to other islands, which suggests that relatively high population densities (see section 5.1.2) and west-to-east entrainment (see section 5.0.5) could be promoting disproportionate contributions of centrally located populations to immigrant pools. More thorough source-tracking of immigrating postlarvae will be necessary to determine the extent to which any or all of these factors drives dispersal asymmetries across the archipelago.

Though immigrant pools can potentially draw from populations across the archipelago, there are telltale signatures that among-island dispersal has little influence on local population dynamics (Hodges and Allendorf 1998). The genetic diversity of *A. stamineus* and *S. stimpsoni* is lower in degraded watersheds, for example, and goby densities are depressed across Oahu, even in remote forested watersheds (see section 5.1.2). If local population dynamics were strongly influenced by among-island dispersal, then recruitment from mixed immigrant pools would likely buoy levels of genetic diversity in degraded waterways (Waits et al. 2008) and sustain population densities in remote watersheds on Oahu. Among-island propagule pressure (i.e., the number and frequency of larvae dispersing among islands) does not appear to be high enough to rescue local populations from watershed or island-wide degradation, which suggests that local populations are more susceptible to local environmental impairment than is now thought. As conditions on Oahu suggest, local populations are likely prone to extirpation because impairment diminishes adult population size and reduces the proportion of locally produced larvae in immigrant pools. Management strategies might therefore prioritize approaches that improve the integrity and productivity of local populations, such as targeted restoration of in-stream habitat, but addressing conditions on Oahu will likely require more inclusive approaches that account for conditions across the island (Cook et al. 2009) and the availability of resources on other islands.

Management of oceanic island stream ecosystems might still have to account for the susceptibility of native fishes to degradation elsewhere in the archipelago, especially in areas that can serve as nurseries for translocation programs. Reconstituting extirpated populations on Oahu, for example, will likely require relocating individuals from robust source populations elsewhere in the archipelago. Thus, favorable stream conditions for maturation and reproduction must be sustained in both source and receiving watersheds. Efforts to reconstitute populations of *Neritina granosa* (a native amphidromous mollusk) illustrate the conditions that restoration efforts might have to account for on Oahu. Restocking of *N. granosa* in streams harboring at-risk populations on Maui have largely involved among-watershed translocations (Skippy Hau, personal communication). But because *N.granosa* is rare across the island, restocking of *N. granosa* on Oahu has involved transplantation from undisturbed streams on Molokai (Bebler and Foltz 2004). This work reflects studies of genetic variation that did not recover evidence of island-specific evolutionary lineages (Hodges and Allendorf 1998, Bebler and Foltz 2004), as well as

evidence indicating that centuries to millennia could elapse before depressed populations recover through natural restocking via larval dispersal from populations on other islands. Hodges and Allendorf (1998) estimated that natural restocking would occur at a rate of approximately 7 migrants per generation, which is consistent with population surveys on Oahu indicating that densities of native species have remained depressed for at least three decades (Luton et al. 2005). Genetic estimates of immigration for native fishes are higher than those for *N. granosa*, but the estimates are nonetheless small enough to call in to question strategies to recover at-risk populations on Oahu by relying on natural restocking or translocation from other watersheds on the island. Thus it might be necessary to manage some watersheds, such as those on the windward coast of Molokai, as resources for recovering populations on Oahu and elsewhere.

Recommendations to temper reliance on among-island dispersal for recovery of at-risk populations is a departure from the prevailing notion that local populations of native freshwater fishes can be rescued by immigration from populations elsewhere in the Hawaiian archipelago (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998). This hypothesis, which largely originated from evidence of weak genetic differentiation and high gene flow among islands (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998, Bebler and Foltz 2004) does not account for estimates of immigration relative to local population sizes, nor does it account for evidence of extirpation and persistently depressed populations in the archipelago (Timbol and Maciolek 1978, Luton et al. 2005). A modest amount of immigration can result in signatures of weak genetic differentiation and high gene flow, but nonetheless have little influence on local population dynamics (Hodges and Allendorf 1998). A 'rule of thumb' holds that one or more migrants per generation can result in genetic homogeneity (Miller and Allendorf 1996), but where populations are large or conversely where populations have been extirpated, a correspondingly large amount of immigration is necessary to influence demographic conditions. Accordingly, stewardship and management of oceanic island stream ecosystems should not solely rely on among-island immigration as a tool or strategy for recovery of at-risk populations (Hughes et al. 2007). Greater success will likely come from approaches that promote population recovery by improving propagule pressure originating from local or proximate sources. This could be done by controlling competitive and predatory invasive species or improving the quality and availability of habitat to enhance productivity within and among watersheds. Where species are rare to absent, this could be done through cross-watershed or among-island translocation.

5.0.3 Otolith-based Analysis of Life History Variation and Movement Potential

Life-history characteristics: The mean age of *Awaous stamineus* in this study was 2.4 years (range: 1.3 – 4.5 years), and the mean LLD was 118 days (range: 57 – 248 days). The distribution of LLD was bimodal with modes around 75 days and 155 days. Most fish (> 90%) hatched between November 2005 and August 2008, and metamorphosed into postlarvae between April 2006 and August 2008. There were no clear pulses in birth or settlement dates, and the oldest individual hatched in October 2002 and metamorphosed in February 2003.

Is amphidromy obligate in Awaous stamineus? Only 38% of the 325 samples for which we could confidently profile larval chemistry showed a high Sr:Ca ratio that unambiguously indicates an oceanic or estuarine (hereafter "SW") larval habitat (Table 3). The remaining fish (62%) showed no strong Sr:Ca peak in the core, indicating residency in freshwater throughout

the larval phase (hereafter "FW"). Independent analysis by EPMA confirmed the results of LA-ICP-MS in a subset of fish showing high (n = 3) and low (n = 5) Sr:Ca in the larval phase, indicating that these patterns are not an artifact of the depth of otolith ablation by LA-ICP-MS. Furthermore, PCA clearly indicates a chemical difference between the FW larval signatures and post-metamorphic signatures. The elemental composition of otoliths can shift during ontogeny due to physiological changes rather than environmental shifts (Fowler et al. 1995). Both FW and SW larval habitats are signified by positive PC1 values while post-metamorphic stages in FW show negative PC1 values (Fig. 1a) indicating that the FW cores are ontogenetically larval and that over- or under-polishing the samples could not be the genesis of an apparently-FW core signature. However, FW larval and post-metamorphic profiles did not differ significantly on PC2 or PC3. Thus, principle components analysis (PCA) revealed a combination of larval (PC1) and freshwater (PC2 and PC3) microchemical patterns confirming that a large percentage of larvae reside in freshwater between hatching and metamorphosis. These patterns of concordant variation in PC1–PC3 indicate that *A. stamineus* in Hawaii are not obligately amphidromous, contrary to previous understanding.

The relative proportions of fish showing FW and SW dispersal histories varied among islands (33 – 44% SW) and watersheds (0 - 100% SW; Table 3). However, the frequency of amphidromy did not differ among islands (ANOVA: $F = 0.86$, $df = 4$, $p = 0.500$), nor did any individual watershed differ significantly from the mean across all others on the same island (z tests: all $p > 0.050$). Though our small samples sizes at the watershed level preclude statistical comparisons at finer spatial scales (Table 3), there were no significant differences in the frequency of amphidromy between the windward and leeward sides of islands (z-test: $p = 0.480$), or watersheds that have estuaries or embayments compared to those that discharge directly to the sea (z-test: $p = 0.290$).

Alternative marine dispersal environments? *A priori* geographic groupings of SW larval chemistry revealed significant differences among islands and watersheds ($MANOVA_{Islands}$: $F_{6,24} = 4.2$, $p < 0.001$; $MANOVA_{Watersheds}$: $F_{6,168} = 1.8$, $p < 0.001$). However, there was no evidence of increasing chemical differentiation among islands with distance (Mantel: $r = -0.45$, $p = 0.940$), as might be expected if larvae disperse by diffusion and the archipelago presents a directional gradient in marine chemistry. Furthermore, *a priori* groups were not strongly supported by DFA.

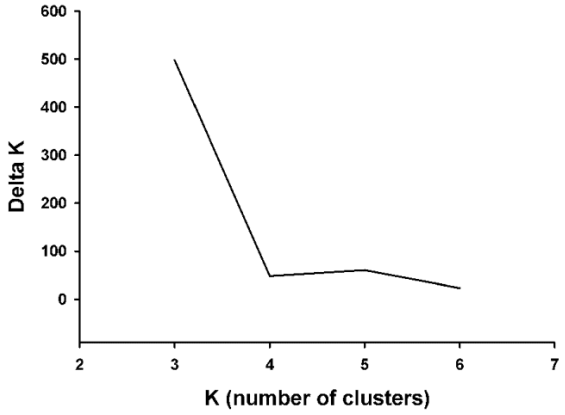

Figure 20: Results of the Delta K information criterion for determining the "true" number of *K* groups in the marine core chemistry data. The highest value of Delta K indicates the most likely *K* groups in the data.

Classification accuracy was moderate for islands (60%) and poor for watersheds (37%), indicating that these geographic groups are not reliable predictors of variation in SW chemistry. Nonetheless, naïve Bayesian clustering of SW larval chemistry revealed evidence of natural groups. All values of *k* greater than one (i.e. $k = 2$-6) were significantly more probable than the null hypothesis of $k = 1$ ($P < 0.010$). Likelihood values increased with the number of groups (Figure 20), but the Delta *k* information criterion indicated that $k = 3$ yielded the largest change in likelihood and had

the lowest variance in likelihood among model runs (Figure 20). We therefore considered $k = 3$ to be the most parsimonious number of clusters, and used it for all subsequent comparisons.

The three Bayesian SW groups were highly significantly different from each other in chemistry ($MANOVA_{Cluster}$: $F_{6,18} = 16.0$, $p < 0.001$; Figures 21 and 22). Furthermore, DFA showed high classification accuracy for all three groups (84 – 96%). The SW clusters showed gradients of both Cu and Sr concentrations across the three groups. SW1 had significantly greater concentrations of Sr and lower concentrations of Cu than other groups. SW3 had significantly greater concentrations of heavy metals (Cu, Zn) than other groups, and significantly lower concentrations of Sr than SW1, whereas SW2 had intermediate concentrations of Cu, and significantly lower Sr than SW1 (all t-tests: $p < 0.001$; significant after sequential Bonferroni correction). The three SW clusters also differed significantly from FW larval and post-metamorphic chemistries in the concentration of most elements (Figure 22). PCA discriminated among the SW groups, FW larval chemistry and post-metamorphic chemistry. PC1 (27% variance explained; Fig. 1a) predominantly separated larval and post-metamorphic chemistry. PC2 (21%) and PC3 (16%) separated the SW larval groups from each other as well as from FW larval and post-metamorphic microchemistry (Figure 22).

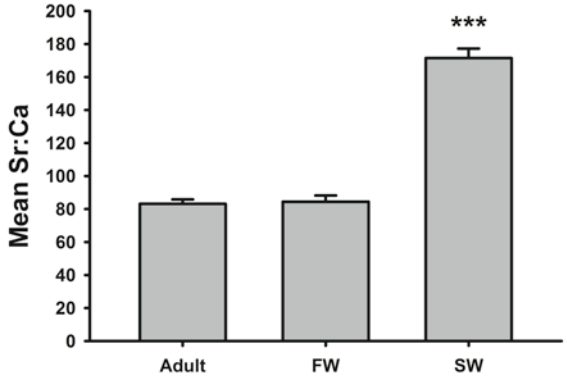

Figure 21: Sr:Ca ratios for post-metamorphic (adult) and putatively freshwater "FW" larvae and marine "SW" larvae. Error bars are standard error. *** $p < 0.0001$

There were no significant differences among islands in the proportion of fishes from each of the three Bayesian SW larval groups (pairwise z-tests: all $p > 0.150$). Sample sizes at the watershed level were too small to allow for any statistical comparisons within islands. There were no significant differences between the windward versus leeward sides of islands (z-tests: all $p > 0.200$), or based on the presence of estuarine habitats (z-test's: all $p > 0.250$). However, larvae in the SW3 group, which featured the highest copper concentrations, exhibited a significant correlation between copper in the marine larval phase and the stream-dwelling post-metamorphic phase ($r^2 = 0.43$, $p = 0.001$; Figure 23), suggesting near-shore residency of SW3 larvae. There was little evidence of such a correlation in the other two clusters of marine larvae (SW1: $r^2 = 0.03$, $p = 0.380$; SW2: $r^2 = 0.08$, $p = 0.070$).

***Individual quality and dispersal history*:** There was no difference in the average age of fish with different larval dispersal strategies (mean: SW = 881 d, FW = 890 d; t = -0.19, p = 0.560). Hatching and metamorphosis dates did not differ significantly between fishes with SW and FW larval chemistries (K-S_{Hatch}: $d$max = 13, k = 67, n = 162, $p = 0.050$; K-S_{Settle}: $d$max = 8, k = 67, n = 162, $p > 0.050$; Figure 24), nor among the three SW groups (all $p \geq 0.050$). However, fishes of SW and FW larval histories did differ in the means and frequency distributions of individual traits. Fishes that went to sea as larvae had significantly shorter larval life durations (K-S_{LD}: $p < 0.001$; means: SW = 108d, FW = 122d; t = -2.31, p = 0.020); they tended to metamorphose at a smaller size (K-S_{SS}: $p < 0.001$; means: SW = 2.39 mm, FW = 2.49 mm; t = -1.87, p = 0.060); and they exhibited significantly faster larval growth rates (K-$S_{Lgrowth}$: $p < 0.001$; means: SW =

0.053 d^{-1}, FW = 0.044 d^{-1}; t = 2.67, p < 0.010). Also, fish that went to sea as larvae tended to have faster post-metamorphic growth rates ($KS_{PSgrowth}$: p < 0.001; Figure 23) although the means were not different (mean: SW = 0.0025 d^{-1}, FW = 0.0023 d^{-1}; t = 1.17, p = 0.240). There were no significant differences in any individual traits among fish with different SW dispersal histories (i.e., SW1, SW2, SW3), but sample sizes were small.

Discussion: Microchemical analyses of otoliths from *Awaous stamineus* revealed multiple dispersal histories among larvae recruiting to watersheds across the Hawaiian archipelago. The most striking result is that amphidromy is not obligate in *A. stamineus*; to the contrary, a majority of larvae remain in or near their natal stream throughout their life. Those larvae that did go to sea showed three distinct marine chemistries, providing evidence of the scope for dispersal variation even within an amphidromous life history. In connecting this diversity of dispersal pathways with longer-term life history traits, we found that larval growth and development rates were enhanced by marine dispersal. The ubiquity of both freshwater and marine strategies across the species range suggests an intriguing balance between the costs and benefits of alternative dispersal strategies in *A. stamineus*.

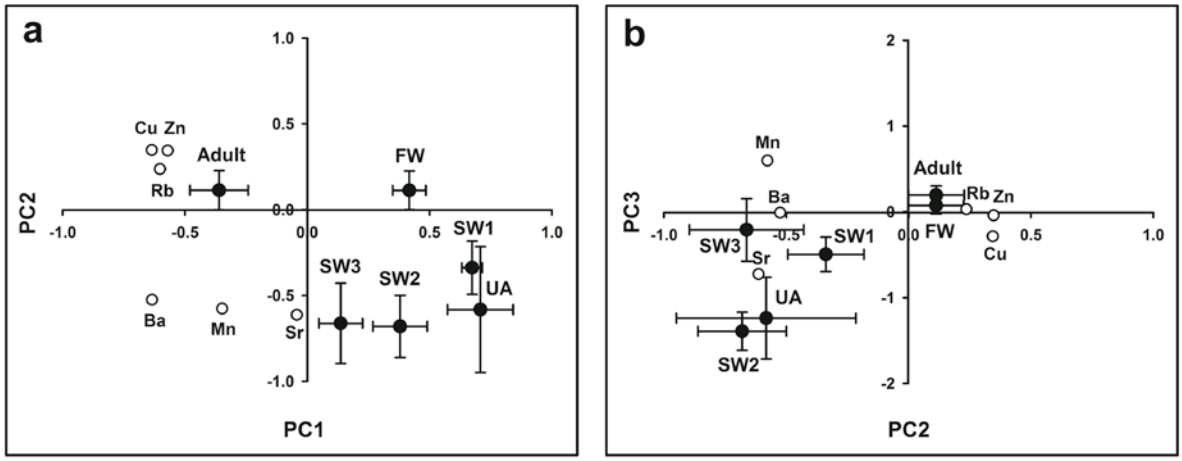

Figure 22: Principle components analysis (PC) showing the orientation of the different chemical groupings in PC space; a) PC1 vs. PC2; b) PC2 vs. PC3. PC1 explains 27% of the variation in chemistry, PC2 explains 21% and PC3 explains 16% of the variation. Filled circles are the mean PC values for each signal type, error bars are 95% confidence intervals; open circles indicate the PC loading for each of the six elements used to generate the PC plots. "FW" is freshwater larval chemistry signals; "SW1", "SW2", "SW3" are the three marine larval clusters identified by naïve Bayesian analysis; "Adult" is post-metamorphic otolith chemistry, representative of stream chemistry; UA are individuals that were unassigned to one of the three Bayesian clusters.

Until now, *A. stamineus* and most other putatively amphidromous gobies were thought to have an obligate marine larval period (McDowall 2010). Hatchlings of amphidromous goby species experience high mortality if exposed to freshwater for extended periods (Iida et al. 2010) providing indirect evidence that individuals must complete a marine dispersal phase to survive. However, direct evidence of facultative amphidromy has been shown in other species of Gobioid fishes, suggesting that obligate amphidromy cannot be assumed, even for species in the same taxonomic family (e.g., Michel et al. 2008).

Several mechanisms could explain the origin and maintenance of amphidromous and non-amphidromous phenotypes in *A. stamineus* and other species. Loss of amphidromy could be the

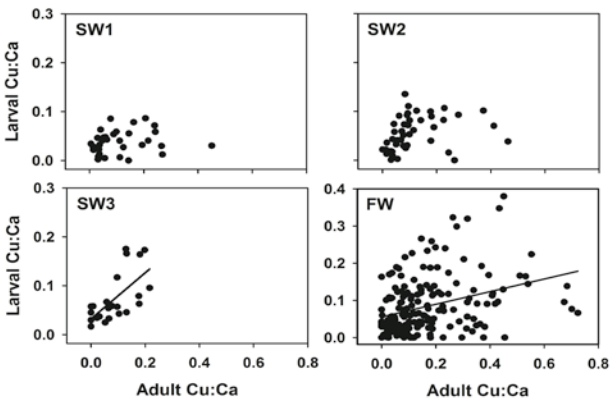

Figure 23: Correlations between post-larval (adult) and larval Cu:Ca concentrations for 4 larval types; FW, SW1, SW2, SW3.

result of the isolation of populations from the ocean by physical barriers or distance, accompanied by genetic differentiation (Michel et al. 2008). Isolation does not appear to be the mechanism giving rise to amphidromy-loss in Hawaiian *A. stamineus* as there is no evidence of strong genetic isolating barriers across the archipelago (Chubb et al. 1998, Lindstrom et al. 2012; see section 5.0.2), and the two life history phenotypes are ubiquitous across the species range. Given that the Hawaiian archipelago must have been colonized by individuals with a marine phase, one might infer that evolution of non-amphidromy must have arisen within the archipelago. However, non-amphidromy also could be ancestral to the Hawaiian populations, perhaps as a recessive trait carried via marine dispersal from founding populations located in Guam or elsewhere in the Indo-Pacific (Lindstrom et al. 2012). This could be addressed by identifying whether populations of *Awaous guamensis* (the closest sister species of *A. stamineus*; Lindstrom et al. 2012) also show facultative amphidromy.

A genetic basis for phenotypic variation is, however, not certain. The polymorphism possibly represents a plastic phenotype dependent on environmental or individual conditions (Bonte and De la Pena 2009). This hypothesis could be addressed by identifying conditions that may promote amphidromy (e.g., variable stream flow conditions). Preliminary evaluation of environmental variation among our sampling sites does not suggest any obvious environmental correlates, but more rigorous tests would be needed to determine whether amphidromy is fixed or plastic at the individual level. Additional comparative studies are also warranted to determine whether facultative amphidromy is exhibited in other amphidromous fauna in Hawaii and elsewhere (Michel et al. 2008).

We found evidence that at least three different marine environments are occupied by fish that went to sea as larvae. The marine dispersal histories identified by Bayesian clustering were distributed across most of our sampled populations, indicating that each marine environment is present across the species' range (Table 3). The most plausible explanation for the observed marine chemical structuring is nearshore versus offshore residence of ocean-going larvae. This mechanism has been identified as an explanation for otolith chemical variation among larval cohorts of at least one marine fish species (Hamilton et al. 2008). Metals such as Pb and Cu are linked with anthropogenic sources in the marine environment, hence high concentrations of these metals in otoliths indicate near-shore residency of larvae (Stauber et al. 2005, Hamilton et al. 2008, Forrester and Swearer 2002). The three marine clusters all differed significantly from each other in concentrations of Cu and Sr. SW3 had high Cu, low Sr; SW1 had low Cu, high Sr, and SW2 was intermediate for both. Furthermore, stream and marine Cu:Ca were correlated for fishes experiencing the SW3 environment, suggesting that the SW3 larvae resided near the stream they recruited to, perhaps in an estuary or in the discharge plume of the stream (Sorenson and Hobson 2005). These findings suggest that the three clusters represent differences in the

distance of dispersal and duration spent in inshore versus offshore waters, where SW3 larvae were retained near-shore, SW1 larvae resided primarily offshore and perhaps transported long distances, and SW2 larvae spent some time near-shore and some offshore (i.e., entrained).

Several other hypotheses have been put forth that could explain chemical structuring in the marine environment, but we find no support for these alternatives. First, temporal changes in marine chemistry can create distinct chemical groupings if larvae are at sea at different times or seasons (Gillanders 2002). We found that *A. stamineus* larvae hatched over a protracted period of time with no distinct seasonal patterns, and no statistical difference in the distribution of hatching or settlement dates among the three marine clusters. Distinct spatial variation within the islands could be generated by the stark climatic differences between the windward and leeward sides of islands. Windward and leeward sides differ in river discharge, driven by orographic precipitation (Giambelluca et al. 1986), as well as ocean circulation patterns (Toonen et al. 2011, Figure 7), which could create unique chemical environments and act to retain larvae on either side of the islands. However, we found no difference in the proportions of the three marine clusters between leeward and windward watersheds. This is not surprising as there is no major barrier to dispersal between windward and leeward habitats for marine taxa in the Hawaiian Islands (Toonen et al. 2011, Figure 7). Estuaries or embayments in watersheds could also create spatial structure; both have high water retention which can retain outgoing larval fishes and create unique chemical signatures (Shima and Swearer 2009). Nonetheless, we found no evidence that marine chemical environments were associated with fishes recruiting to watersheds with estuaries or embayments. Excluding these possibilities further supports our inference that SW clusters correspond to a nearshore-offshore pattern of larval residency. This has implications for the degree and spatial scale of connectivity among streams, indicating that population replenishment involves much higher levels of local retention than previously thought (e.g., Chubb et al. 1998).

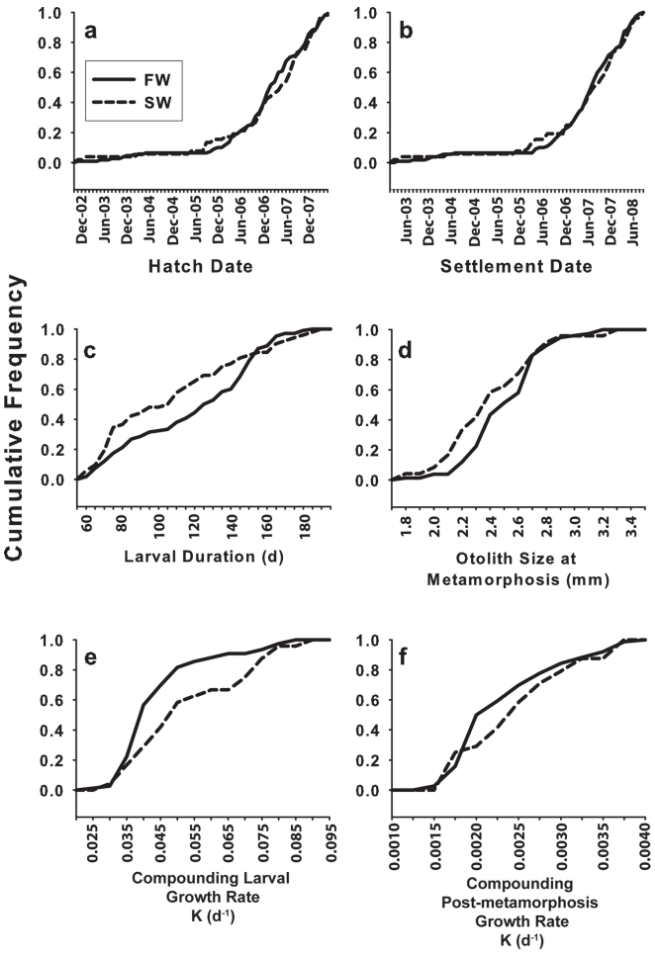

Figure 24: Cumulative frequency curves illustrating the differences between amphidromous (dashed line) and non-amphidromous (solid line) larvae in the distributions of; a) hatch dates, b) settlement dates, c) larval durations, d) otolith radius size at metamorphosis, e) compounding daily larval growth rate, and f) compounding daily post-metamorphosis growth rate. Compounding growth rates were estimated from the von Bertalanffy growth equation.

Amphidromy has clear potential costs associated with the marine larval phase, including risk of predation, physiological costs of adjusting to the marine environment, energy costs of locomotion in the pelagic zone, and the challenge of finding the way back to extremely patchy stream habitats (McDowall 2010). These costs could be mitigated by reducing the length of the marine dispersal period, or by reducing the distance of dispersal from the natal habitat either by taking advantage of currents or by active natal homing (McDowall 2010). Avoidance of a marine phase altogether is the most extreme form of limiting dispersal costs, and appears to be a favorable strategy for *A. stamineus*. Furthermore, given our findings of differences in isotope signatures among ocean-going larvae, we surmise that active near-shore retention may be a mechanism used by some marine larvae to reduce dispersal distance. This raises the question of why amphidromy is retained at all following colonization of productive tropical island streams (Bonte and De la Pena 2009, Bonte et al. 2012).

It appears that marine larval dispersal, at least for *A. stamineus*, has benefits that balance against the costs, leading to a persistent polymorphism via unknown processes of frequency dependence. We found that larval dispersal through the marine environment was associated with enhanced larval condition and post-metamorphic condition, which likely translates in to increased lifetime fitness. Fishes that went to sea as larvae spent significantly less time as larvae, tended to metamorphose into postlarvae at a smaller size, and grew significantly faster during the larval period compared to fishes that stayed in the streams as larvae. Larval growth advantages have been demonstrated to continue shortly following metamorphosis (Sponaugle et al. 2006), but our results suggest that these advantages can continue for years. We found that fish that went to sea as larvae also tended to have faster post-metamorphic growth rates than non-amphidromous fish (although mean growth rate was not significantly different) despite co-experiencing the same stream environment for most of their lives. The distinctions we found between FW and SW life histories are not wholesale differences in the range of values of these traits, but rather significant shifts in the frequency distribution. Each of these traits has been shown to be related to larval survivorship (Searcy and Sponaugle 2001), early post-metamorphosis condition (Hamilton et al. 2008), early post-metamorphosis survivorship (Sponaugle et al. 2006), adult survival (Goater 1994), longevity and reproductive success (Taylor et al. 1998) in a number of disparate taxa including fishes, amphibians and insects. These traits also have been previously linked to dispersal histories in fishes and insects (Weiss et al. 1987, Shima and Swearer 2009).

The evidence for long-lasting benefits of a marine larval experience suggests the possibility of an influence on lifetime fitness through reproductive advantages. We found that the mean ages were not different between fish that experienced either a freshwater or marine larval period. Presumably then, the faster growing amphidromous fishes would reach size dependent sexual maturity earlier; assuming the same size at maturity, this could impart a fitness advantage for fast growing, amphidromous individuals. The possible fitness advantage from faster growth of marine larvae presumably selects for marine dispersal, balancing against the potential benefits of larval retention. This balance could serve as an evolutionary mechanism for the maintenance of a dispersal bet-hedging strategy (e.g., Krug 2009). Remaining in a stream as a larva reduces mortality (i.e., predation, becoming lost at sea) and energetic costs (e.g., physiological acclimatization, locomotion) associated with marine dispersal. However, the benefits imparted by marine life (i.e., faster life-time growth rate) may offset these individual costs so that the amphidromous life history strategy is not lost over evolutionary time scales. Our analysis highlights the opportunities to use measures of individual history and performance to assess the

selective costs and benefits at play in generating and maintaining intraspecific variation in dispersal.

The dispersal polymorphism discovered in *A. stamineus* could have far reaching consequences for the ecology and evolution of populations. Dispersal among sub-populations can promote demographic and genetic stability of sub-populations and metapopulations (Holland and Hastings 2008, Walter et al. 2009). Amphidromy undoubtedly facilitates dispersal of *A. stamineus* among watersheds. An offshore larval phase (i.e., SW1) can enable long-distance dispersal, connecting populations among islands or even disparate archipelagos. This seems likely in *A. stamineus* because the average larval duration is 118 days, and genetic studies indicate that between-island dispersal is likely common (Chubb et al. 1998, Lindstrom et al. 2012, and see section 5.0.2). While only ~40% of fish disperse at sea, this appears to be enough to sustain a high degree of contemporary connectivity (Chubb et al. 1998, Lindstrom et al. 2012, and see section 5.0.2). Depending on the size of the propagule pool, marine dispersal may be sufficient to buoy disturbed and declining populations (Walter et al. 2012). However, the demographic benefits of dispersal for *A. stamineus* are likely limited to local- or island-scales as most amphidromous larvae are either retained near-shore or entrained.

While dispersal can contribute to persistence of local populations and of metapopulations, local retention of some larvae can also promote long-term persistence of metapopulations (Hastings and Botsford 2006) through the replacement of local breeding stock (Botsford et al. 2009). In the case of *A. stamineus* across Hawaii, most larvae stay within the natal stream throughout development, and another 9% on average stay near-shore (i.e., SW3), close to their recruitment stream, possibly recruiting to their natal stream (i.e., self-recruitment). However, we found that the proportions of locally recruiting larvae varied widely at the scale of individual streams. The rates of local recruitment (through non-amphidromy and retention of marine larvae) here are concordant with some of the highest estimates of self-recruitment for marine species with dispersive larvae (Almany et al. 2007, Hogan et al. 2012). Most previous studies documenting rates of local retention were conducted at local-scales relative to the scale at which the populations are interacting via connectivity. This study is among the first to examine rates of self-recruitment and dispersal for populations across an entire species' range, providing a comprehensive perspective on how processes that determine connectivity and population replenishment within a species can be spatially variable. Our findings illustrate the importance of sampling intensively and broadly to achieve robust estimates of self-recruitment and dispersal for a metapopulation or species.

Variation in dispersal strategies has equally profound consequences for the conservation of amphidromous species. Oceanic island streams are severely threatened by habitat alteration, invasive species, water abstraction, and drying due to climate change (Brasher 2003, Walter et al. 2012). Successful conservation actions rely on an adequate understanding of the biology of the focal species, especially when management efforts can be contentious. For amphidromous species, preserving ocean-stream connectivity and remediating downstream habitats (i.e., at or near the stream mouth) would likely provide the greatest benefits if species are obligately amphidromous (Walter et al. 2012). In this scenario, local populations would be open to immigration, and declining populations could rely on rescue from distant sources. However, local processes would be expected to play a much larger role in population persistence under conditions of facultative amphidromy where local recruitment is common. Populations would

likely benefit most from improving in-stream conditions such as habitat alteration, natural flow regimes and invasive species control (Brasher 2003, Walter et al. 2012). At present, we do not know whether the observed patterns of variation in self-recruitment and dispersal are temporally stable. Stream conditions in Hawaii (and on oceanic islands in general) can be highly variable, potentially creating a temporally dynamic balance of self-recruitment and condition-dependent dispersal, where in some years marine-mediated dispersal is favored but otherwise non-amphidromy wins the day. Further comparisons will be necessary to determine the full range of life history flexibility in *Awaous* and other amphidromous species that occupy temporally-variable and spatially-patchy environments.

5.0.4 Use of Oxygen Isotopes in Otoliths for Reconstructing Life History

Age and growth: The average age of the nine individuals that were examined was 153 days (range: 132 – 165 d; CV = 0.08; Table 7) and average larval duration was 120 d (range: 105 - 135 d; CV = 0.08; Table 7). There was extensive variation observed in the larval otolith radius (LOR; the distance from primordium to metamorphic mark) among individuals, which we used as a proxy for size at metamorphosis (mean: 231 µm; range: 145 – 283 µm; CV = 0.19). Postlarval growth (PLG) rates were significantly higher than mean larval growth (MLG) rates (PLG: mean = 3.51 µm/d; MLG: mean = 1.91 µm/d; t = -2.94, df = 16, $p < 0.01$; Figure 25). However, growth within the larval period was not constant, and each individual clearly demonstrated a fast growth period early in larval life (ELG) when compared to late larval life (LLG). The ELG was a period of significantly faster larval growth than the LLG (ELG: mean = 3.29 µm/d; LLG: mean = 1.55 um/d; $t_{(16)} = 12.77$, $p < 0.01$; Figure 25). Interestingly, the ELG growth rate was similar to the maximum PLG rate ($t_{(16)} = -0.40$, $p = 0.69$), so in most cases it exceeded the PLG rate (Table 7, Figure 25). The duration of the ELG anomaly (ELGD) varied widely among individuals (mean: 28 d; range: 18 – 39 d; CV = 0.22; Table 7, Figure 26).

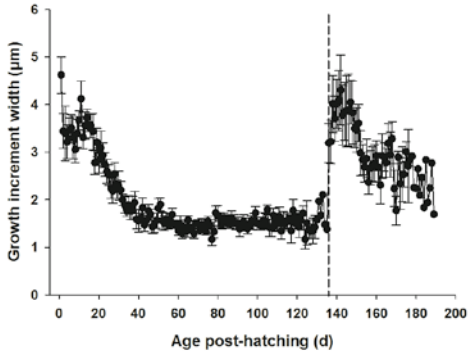

Figure 25: The daily growth profile for nine *Awaous stamineus*. Dots indicate mean daily growth increments; error bars are standard deviations. The vertical dashed line indicates the transition from larval to postlarval life growth periods.

We found that growth performance during the ELG period did not always predict future performance and outcomes according to metrics reflecting size at metamorphosis and postlarval growth rate. The ELGD was not significantly correlated with either size at settlement (LOR: $r^2 = 0.02$, $p = 0.71$) or postlarval growth rate (PLG: $r^2 = 0.06$, $p = 0.53$). However it was negatively correlated with late larval duration, the period of larval growth remaining following the end of the early larval growth period ($r^2 = 0.80$, $p < 0.01$; Figure 27). The slope of this relationship indicates that every day of fast growth during the ELG period reduces slower growth during the LLG period by two days. Larvae that experienced extended early growth anomalies therefore exhibited significantly shorter larval durations.

***Otolith elemental and isotopic chemistry*:** Both Sr:Ca and $\delta^{18}O$ profiles indicated a major shift in environmental chemistry at or around the point of metamorphosis (Figures 4 and 28). Sr:Ca ratios were high during the larval period and declined at or around the metamorphosis mark, typically by an order of magnitude (Figure 28). This pattern reflects individuals leaving a high salinity environment (larval Sr:Ca mean = 300) and entering a low salinity environment (postlarval Sr:Ca mean = 119; $t = 7.32$, $df = 517$, $p < 0.01$). We also found that $\delta^{18}O$ shifted downward at metamorphosis by 4-5‰, indicating an abrupt change from an ^{18}O rich

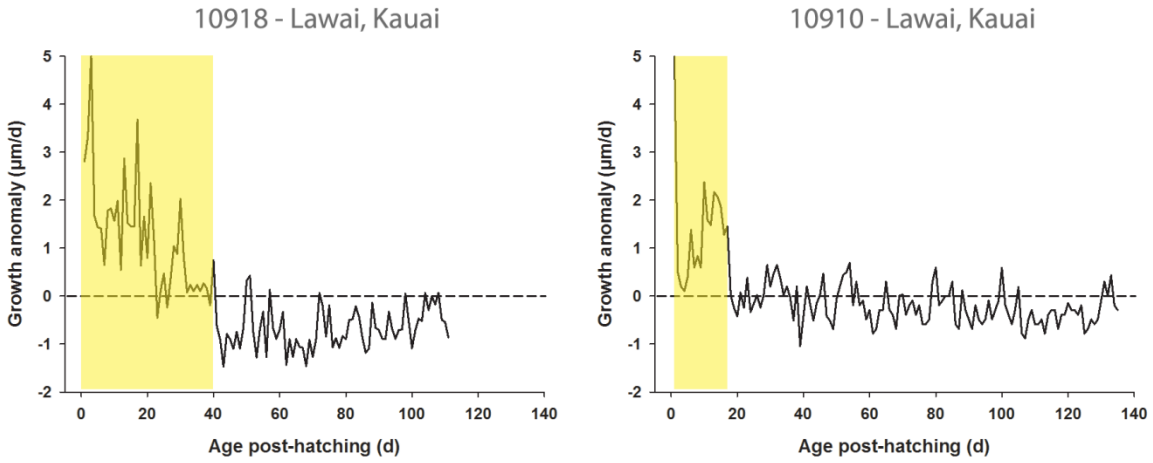

Figure 26: Early larval growth anomalies. Examples of larval growth anomalies are presented for two individuals representing the longest and shortest duration anomalies in the dataset. Horizontal dashed lines indicate mean larval growth rate (MLG) across the entire larval period. Early larval growth anomalies were defined as sustained growth rates (more than 3 consecutive days) above the MLG, where d = days and µm = micrometers

environment to an ^{18}O depleted environment (Figure 28). In all cases, the $\delta^{18}O$ shift occurred precisely at the metamorphosis mark, compared with the shift in Sr:Ca which did not always occur at the metamorphosis mark (Figure 28). Larval and postlarval environments also differed significantly in several other elements including Mn, Cu, Zn, Ba and Pb, all of which were significantly higher in concentration in the postlarval environment compared to the larval environment (all $t > -2.12$, all $p < 0.03$).

During the larval growth periods, $\delta^{18}O$ was surprisingly stable from hatching to metamorphosis, rarely varying more than 1‰ and often varying less than the precision of the ion microprobe (0.3-0.6‰). The general trend in $\delta^{18}O$ among individuals was either stable $\delta^{18}O$ or a slight increase of ~ 1‰ from hatching to metamorphosis. However, trace element concentrations from LA-ICP-MS varied significantly within the larval period. The early larval growth periods had significantly lower concentrations of Ba ($t_{(16)} = -2.20$, $p = 0.04$) and Mn ($t_{(16)} = -2.31$, $p = 0.03$) than the late growth periods. The ELG also had higher average concentrations of Sr relative to calcium than the LLG (ELG mean: 356; LLG mean: 256; $t_{(16)} = 1.79$, $p = 0.09$) although this difference did not translate to statistically significant differences in Sr:Ca ratios due to variation within the ELG among individuals. Interestingly, variation among individuals in some trace element ratios within the ELG was correlated with the duration of the ELG anomaly period. Individuals with longer periods of fast growth in the ELG had lower concentrations of Sr:Ca during the ELG compared to individuals with shorter ELG periods ($r = -0.74$, $p = 0.02$; Figure 29). Only one other element (Zn) was correlated with ELG duration ($r = -0.68$, $p = 0.04$), but

visual analysis of the trend suggests that the relationship is driven by two outlier points. Variation in $\delta^{18}O$ was not correlated with ELG duration.

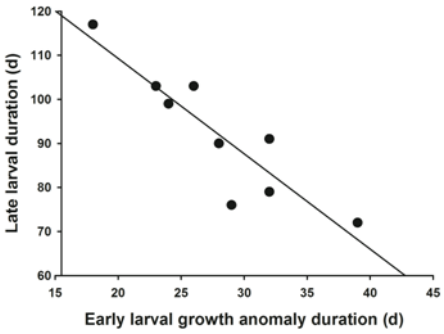

Figure 27: Correlation between early larval growth anomaly duration and late larval duration. Solid line indicates best fit regression line.

Within the postlarval period, both trace element chemistry and $\delta^{18}O$ varied considerably within individuals. Trace elements varied the most within individuals; Sr was the least variable and Pb was the most variable (CV range: 0.18 – 4.79). As much as 2‰ variation was found for $\delta^{18}O$ (CV = 0.07) within individuals. This indicates the relative chemical instability of the postlarval freshwater stream environment compared with the larval marine environment. Both trace elements and $\delta^{18}O$ varied among watersheds as well. We found that $\delta^{18}O$ varied by ~1.3‰ (CV = 0.02) among watersheds, though trace elements were more variable among watersheds (CV range: 0.47 – 1.33) than $\delta^{18}O$. Interestingly, the one watershed (Waimea, Oahu) for which we had $\delta^{18}O$ measurements from two individuals showed consistency between individuals. The averages differed by only 0.05‰, indicating that $\delta^{18}O$ is a consistent measure of the local environment. A comparison of postlarval mean $\delta^{18}O$ values to stream water temperature measured at the time of sample collection recovered non-significant negative relationship (slope = -0.22, r = -0.38, p = 0.53).

Fish ID	Island	Watershed	Date collected	Hatching date	TL (mm)	Age (d)	LD (d)	PM (d)	LOR (µm)	MLG (µm/d)	ELG (µm/d)	LLG (µm/d)	ELGD (d)	PLG (µm/d)
10166	Molokai	Halawa	4/23/2011	11/9/2010	24	165	123	42	200	1.63	2.66	1.26	32	3.12
10910	Kauai	Lawai	5/17/2011	12/6/2010	25	162	135	27	225	1.67	3.07	1.48	18	4.19
10912	Kauai	Lawai	5/17/2011	12/7/2011	24	161	123	38	283	2.12	3.5	1.77	24	2.58
10918	Kauai	Lawai	5/17/2011	12/26/2010	24	142	111	30	269	2.42	3.68	1.77	39	3.44
10919	Kauai	Lawai	5/17/2011	12/30/2010	24	138	105	33	145	1.9	3.08	1.45	29	4.39
11455	Oahu	Waimanalo	6/2/2011	12/19/2010	26	165	111	54	201	1.81	3.05	1.31	32	2.59
11527	Oahu	Kaluanui	6/7/2011	1/4/2011	21	154	129	25	247	1.91	3.27	1.57	26	2.32
11542	Oahu	Waimea	6/8/2011	1/27/2011	26	132	118	14	261	2.21	3.62	1.77	28	7.04
11543	Oahu	Waimea	6/8/2011	1/3/2011	22	156	126	30	249	1.98	3.72	1.59	23	1.92
Mean					**24**	**153**	**120**	**33**	**231**	**1.96**	**3.29**	**1.55**	**28**	**3.51**
CV					**0.07**	**0.08**	**0.08**	**0.35**	**0.19**	**0.13**	**0.11**	**0.12**	**0.22**	**0.44**

Table 7: Age and growth statistics for nine *Awaous stamineus* collected for otolith microstructure analysis. TL = total length; LD = larval duration; PM = post-metamorphosis age; LOR = larval otolith radius, used as a proxy for size at metamorphosis; MLG = mean larval growth rate; ELG = early larval growth rate anomaly; LLG = late larval growth rate; PLG = postlarval growth rate; ELGD = early larval growth anomaly duration.

Discussion: Integrating multiple tools and approaches to the study of otoliths, including age and growth analysis of increments, trace element chemistry, and oxygen isotope fractionation can provide insight into the biology of individual fish larvae. Integrated approaches can help to discover patterns of spatial and temporal variation in habitat use and reveal the consequences of encountering different environments for growth and performance (Shima and Swearer 2009, Hogan et al. in review). We found that analysis of daily otolith growth rings can provide detailed information about individual growth rates, which can influence fitness and survival. We have

also shown that coupling growth data with trace element data from LA-ICP-MS can link performance with environmental conditions associated with habitat use. The addition of fine-scale $\delta^{18}O$ measurements from SIMS offers further understanding of habitat use and environmental variation by providing a level of temporal precision that LA-ICP-MS currently cannot match.

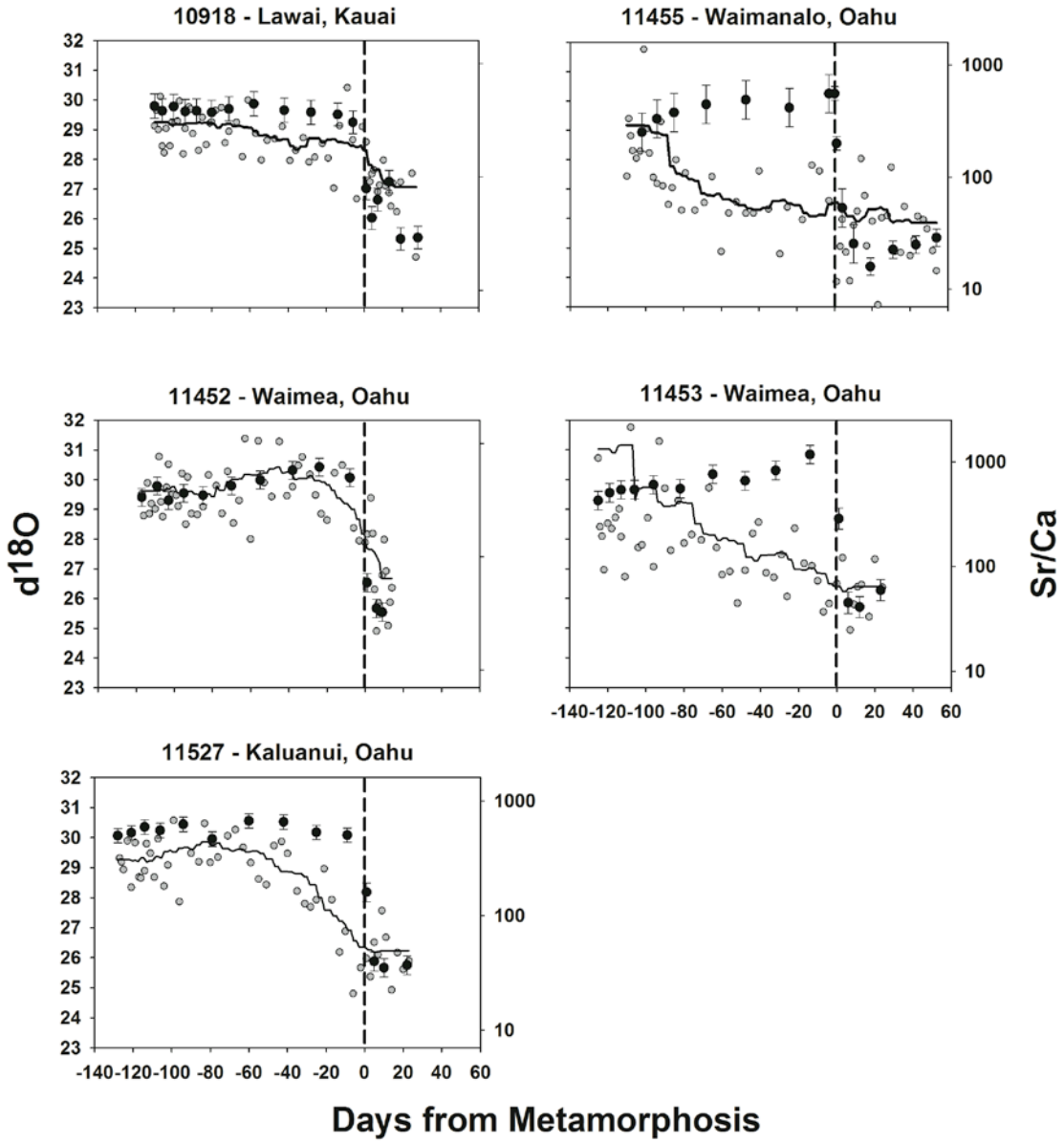

Figure 28: Otolith transect profiles of $\delta^{18}O$ (black circles) and Sr:Ca (gray dots) for five *Awaous stamineus*. Chemistry profiles are centered around the metamorphosis mark (vertical dashed line). $\delta^{18}O$ error bars are standard deviations.

Larval growth rate can strongly influence the probability of survival in fishes by influencing larval vulnerability to size-selective predation (Houde 1997). We found that growth rates of *A. stamineus* were not constant throughout an individuals' life and there was a pronounced period of fast growth early in the larval stage. All individuals exhibited rapid growth during early larval

development, but the duration of this period varied widely among individuals. Variation in growth rate can translate to variation in fitness outcomes including survival and age at first reproduction (Bailey and Houde 1989). Rapid growth during early larval development may be favored by selection because size-selective mortality may decrease and survivorship increase if larvae escape a "window of vulnerability" by reaching a threshold size after which predation risk markedly declines (Cowan et al. 1996). Spending less time in higher-predation risk environments can also increase survivorship (Cowan et al. 1996). We found that individuals with longer periods of rapid early larval growth tended to exhibit shorter larval life durations (Figure 27).The marine larval stage is undoubtedly a period of high mortality; hence individuals with shorter larval life durations likely exhibit greater survivorship.

While mortality and growth-rate are intimately linked through size-selective or habitat-dependent predation, prevailing environmental conditions also can limit growth and survival (Hamilton et al. 2008, Shima and Swearer 2009). We found that the duration of the early larval growth anomaly was correlated with trace element chemistry, specifically Sr:Ca (Figure 29). Although the relationship is not overwhelmingly strong and we have a limited sample size, this finding raises the possibility that particular environmental conditions can influence larval growth rates. Sr:Ca is indicative of salinity environments (Zimmerman 2005), where higher Sr is attributable to higher salinity. We found that ELGD was negatively correlated with Sr:Ca, whereby longer periods of early larval growth were associated with lower salinity environments (Figure 29). Values of Sr:Ca during the period of early larval growth vary considerably among individuals, but the values never reached levels reflecting freshwater conditions. Thus, lower Sr:Ca values during the larval period likely correspond to residence in near-shore environments that receive some freshwater inputs from the stream runoff (Hogan et al., in review). If so, then larvae that remain in near-shore environments likely have a fitness advantage over larvae that occupy off-shore environments. Though further study is needed to confirm this hypothesis, larval habitat use has been linked with performance and survival in other species (Hamilton et al. 2008, Shima and Swearer 2009).

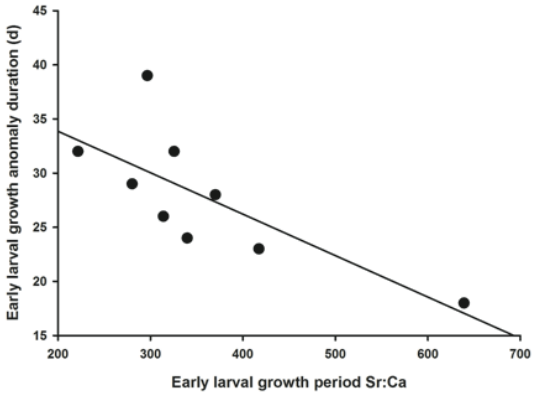

Figure 29: Correlation between Sr:Ca in the early larval growth periods and the duration of the early larval growth anomaly. Solid line indicates best fit regression line.

Trace element chemistry and $\delta^{18}O$ were able to clearly identify the transition of *Awaous* larvae between marine and freshwater environments. Though there is a long tradition of using otolith Sr and $\delta^{18}O$ to study diadromous fish migration (e.g., Casselman 1982, Nelson et al. 1989), this is the first fine-scale comparison of Sr and $\delta^{18}O$ derived from LA-ICP-MS and SIMS. Both Sr:Ca and $\delta^{18}O$ showed marked declines at or around the metamorphosis mark. Shifts in Sr:Ca values, which serve as a tracer of salinity, indicate movement from high to low salinity environments. The observed shifts in $\delta^{18}O$ match the shifts found in Sr:Ca values, which suggests that changes in $\delta^{18}O$ values reflect a transition from highly enriched tropical marine environments to depleted precipitation-driven freshwater streams

(Giambelluca et al. 1986). In all specimens, however, we found that the $\delta^{18}O$ shift occurred precisely at the metamorphosis mark, compared with the shift in Sr:Ca which did not always occur at the metamorphosis mark.

These results suggest that both techniques are effective for determining marine-freshwater transitions. Both LA-ICP-MS and SIMS clearly identified habitat shifts in all samples, so neither method suffers from significant detection error. However, SIMS performed better than LA-ICP-MS at precisely reconstructing migratory transitions. Parallel analyses of Sr:Ca with LA-ICP-MS demonstrated that the approach can match the precision of SIMS in some but not all cases (Figure 28). To date, the coincidence of metamorphosis with the movement from oceanic to stream habitat during larval settlement has been limited to inferences drawn from corresponding trends in otolith microchemistry and microstructure. Analyses of $\delta^{18}O$ with SIMS clearly showed that habitat transitions coincide with metamorphosis from the larval to postlarval form (Figure 28).

LA-ICP-MS results in less precise records than SIMS because the instrumentation generates transects of lower temporal resolution data. SIMS has superior lateral and depth precision compared to LA-ICP-MS, where SIMS has 10 x 3µm beam width x depth and LA-ICP-MS has 25 x 30µm beam width x depth (Pisonero et al. 2009). SIMS has a temporal resolution of single daily growth increments (Weidel et al. 2011), and through individual spot sampling, SIMS also allows for user-selected data collection where individual spots can be staggered and overlapped to sustain or increase temporal resolution. Though beam depth and width represent hard constraints on the resolution of LA-ICP-MS, sampling spots rather than a continuous transect could increase the precision of the approach.

Prior studies suggest that measurement of $\delta^{18}O$ can have the additional benefit of providing information about environmental temperatures (Thorrold et al. 1997). There is a predictable relationship between $\delta^{18}O$ and temperature, where a 0.22‰ increase in $\delta^{18}O$ corresponds to a 1°C increase in temperature (Visser et al. 2003). Though we did not find a strong relationship between $\delta^{18}O$ measured during the postlarval period and stream temperatures measured at the time of capture, the comparison involved a very small data set (i.e., only five data points) with little statistical power to detect a relationship. It is possible, however, that the absence of a relationship is attributable to other factors. First, $\delta^{18}O$ concentrations depend partly on end-member concentrations of ^{18}O, which can correspond to precipitation and groundwater sources in Hawaii (Scholl et al. 1996). The considerable variation in $\delta^{18}O$ end-members that has been found across Hawaiian streams (Coplen and Kendall 2000) could overwhelm variation attributable to temperature. It could be possible to identify temperature differences by characterizing stream water end-members in our sample watersheds. Second, our measures of stream temperature were only single point measures and thus may not be representative of conditions reflected by $\delta^{18}O$ values. More thorough measurement of stream end-member temperatures thus could improve pattern detection. Interestingly, the $\delta^{18}O$ values obtained for the two samples from Waimea (Oahu) were surprisingly similar (Table 8). The samples differed by only 0.05‰, which corresponds to a temperature range of less than 1°C. Additional work is clearly warranted, but this finding suggests that $\delta^{18}O$ could be a useful index of environmental temperatures so long as sufficient data is available on stream end-member values.

We have demonstrated that SIMS does have significant analytical advantages over LA-ICP-MS, but SIMS involves more demanding sample preparation (i.e., in terms of time and precision) and greater monetary costs for analyses. Sample preparation for either SIMS or LA-ICP-MS is not trivial, but SIMS requires additional steps (e.g., otolith roasting) as well as greater attention to sample precision (e.g., narrower tolerances of surface flatness and smoothness). Indeed, minor variation in otolith polishing likely prevented us from capturing the full growth history of each individual. This effectively reduced the size of our data set because individuals with partial records were removed from statistical analyses. It also compounded the monetary cost of data collection using SIMS, which can be significantly higher than LA-ICP-MS. Typical lab fees for an LA-ICP-MS facility are currently $100-150 per hour, whereas using SIMS currently costs $120-300 per hour. Additionally, throughput of SIMS is typically much lower than LA-ICP-MS. An otolith transect in LA-IPC-MS can take between 1-10 minutes depending on scan rate and otolith size; SIMS analysis takes ~3 minutes per spot, with the number of spots analyzed dependent on the size of the otolith. For this study, we sampled ~12 otolith transects per hour with LA-ICP-MS, compared to a single otolith spot-transect every ~2 hours with SIMS. Thus, on a per otolith basis, SIMS ($240-600 per sample) is much more expensive than LA-ICP-MS ($8-10 per sample).

Sample	Island	Watershed	Mg:Ca	Cr:Ca	Mn:Ca	Ni:Ca	Cu:Ca	Zn:Ca	Sr:Ca	Ba:Ca	La:Ca	Pb:Ca	δ18O	°C
10166	Molokai	Halawa	0.18	0.07	0.48	0.02	0.06	0.06	143.89	0.27	0.04	0		21.1
10910	Kauai	Lawai	0.21	0.04	1.57	0.21	0.13	0.1	255.3	0.33	0	0.01		22.7
10912	Kauai	Lawai	0.18	0.04	1.48	0.05	0.01	0.06	84.07	0.69	0.04	0.01		22.7
10918	Kauai	Lawai	0.35	0.09	3.35	0.15	0.38	0.09	121.59	0.56	0.01	0.01	28.51	22.7
10919	Kauai	Lawai	0.32	0.03	1.49	0.02	0.31	0.33	100.34	0.69	0.02	0.03		22.7
Mean	Kauai	Lawai	0.26	0.05	1.97	0.11	0.2	0.14	140.32	0.57	0.02	0.01	28.51	22.7
11455	Oahu	Waimanalo	0.15	0.02	0	0.19	0.24	0.2	39.92	0.25	0.04	0.07	27.76	24.5
11527	Oahu	Kaluanui	0.08	0.01	0.09	0.06	0.1	0.02	49.34	0.1	0.01	0	29.14	22.5
11542	Oahu	Waimea	0.09	0.01	0	0.06	0.07	0.03	94.84	0.09	0.01	0	28.91	24.3
11543	Oahu	Waimea	0.1	0.1	4.04	0.08	0.15	0.28	177.86	1.1	0.06	0.11	28.96	24.3
Mean	Oahu	Waimea	0.09	0.06	2.02	0.07	0.11	0.15	136.35	0.6	0.03	0.06	28.94	24.3
		Mean	**0.19**	**0.05**	**1.45**	**0.09**	**0.16**	**0.13**	**120.75**	**0.47**	**0.02**	**0.03**	**28.63**	**23.1**
		CV	**0.47**	**0.64**	**0.88**	**0.67**	**0.66**	**0.74**	**0.48**	**0.62**	**0.67**	**1.33**	**0.02**	**0.05**

Table 8: Summary of postlarval (stream) chemistry for nine *Awaous stamineus* individuals from five watersheds across the Hawaiian archipelago. All values are the average postlarval measurements from LA-ICP-MS or SIMS. Mean within watershed values are presented for watersheds with multiple individuals. °C indicates the stream water temperature measured at the time of sample collection.

Though some consideration must be given to logistical and cost constraints, parallel analyses of otolith microstructure, microchemistry and $\delta^{18}O$ can be a powerful approach for relating individual performance to environmental history in migratory fishes. Using SIMS alongside LA-ICP-MS can provide unprecedented insight into larval migration and habitat use, especially when microstructural analysis provides information on signature life history events such as metamorphosis. Even a modest number of samples can provide a basis for reconstructing relationships between environment and performance factors (e.g., growth) that can influence survival. Pending further exploratory studies, SIMS may also prove useful for reconstructing long-term records of responses to environmental change in freshwater environments, where $\delta^{18}O$ values serve as a proxy for tracking climate-driven shifts in temperature and corresponding shifts in life history and performance.

5.0.5 Coupled Biophysical Modeling of Larval Dispersal

Dispersal modeling using constant spawning: The majority of larvae that successfully settled on Hawaii originated from Hawaii under both larval life duration (LLD) scenarios. The propagule pressure originating from Hawaii also progressively diminished towards the northwestern regions of the archipelago. For example, under short LLD conditions, the proportion of larvae entering streams on Oahu and Kauai that originated on Hawaii constituted less than 25% of successful settlers (Figure 30). Under conditions of longer LLD, the propagule pressure originating from Hawaii did not diminish as steeply as it did under conditions of shorter LLD. The proportion of larvae entering streams on Oahu and Kauai that originated from Hawaii, for example, consistently exceeded 25% of successful settlers (Figure 30).

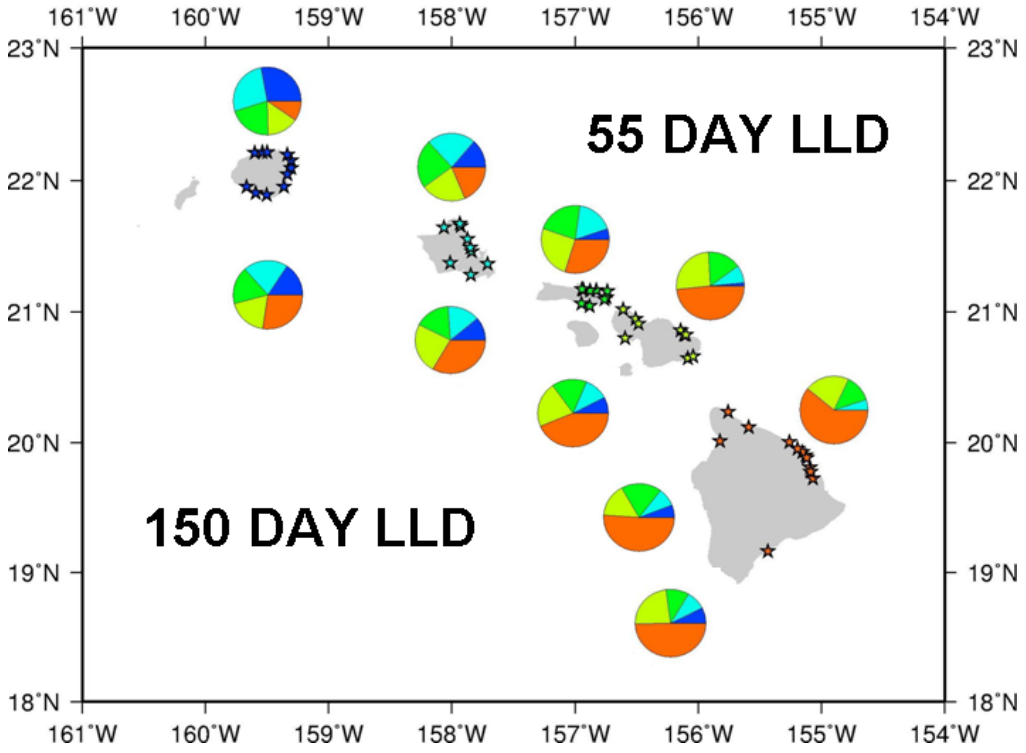

Figure 30: The proportion of successfully settled larval propagules according to their island of origin for Kauai (dark blue), Oahu (light blue), Molokai (green), Maui (yellow) and Hawaii (orange) under conditions of short larval life duration (55 days), and long larval life duration (150 days). Spawning output was kept constant between locations and through time. Stars indicate the location of stream mouths for watersheds where larval propagules entered the oceanic environment.

A clear dispersal asymmetry emerged under both short and long LLD conditions, with larvae predominantly dispersing east to west across the archipelago (Figure 30). Simulations showed that west-to-east dispersal was more limited than east-to-west dispersal. Among-island dispersal of larval propagules was much greater from Hawaii to towards Kauai than in the reverse direction, where propagules originating from Kauai reached Hawaii only under long LLD conditions. The dispersal asymmetry is likely attributable to the southeast to northwest directionality of offshore currents. However, the distribution of release sites- with more sites

being located in the southeastern region of the archipelago- likely also disproportionately elevated the probability of east-to-west dispersal (Figure 30).

The probability matrix for connectivity among watersheds under the two LLD scenarios further illustrates that dispersal is asymmetric and directional across the archipelago (Figures 31 and 32). According to percentages of successfully settled larvae at each receiving site relative to source site, which are generally low (indicating that the vast majority of larvae are lost at sea), the patterns of directional dispersal among watersheds are consistent with among-island trends (Figures 31 and 32). For example, the probabilities of watersheds on Kauai serving as source sites for larvae settling in watersheds on the island of Hawaii are lower than the reverse scenario. Higher connectivity occurs among sites on the island of Hawaii compared to sites on the other islands of the archipelago except for Kauai, where among-watershed connectivity is also relatively high (Figures 31 and 32). The patterns of higher connectivity around the islands of

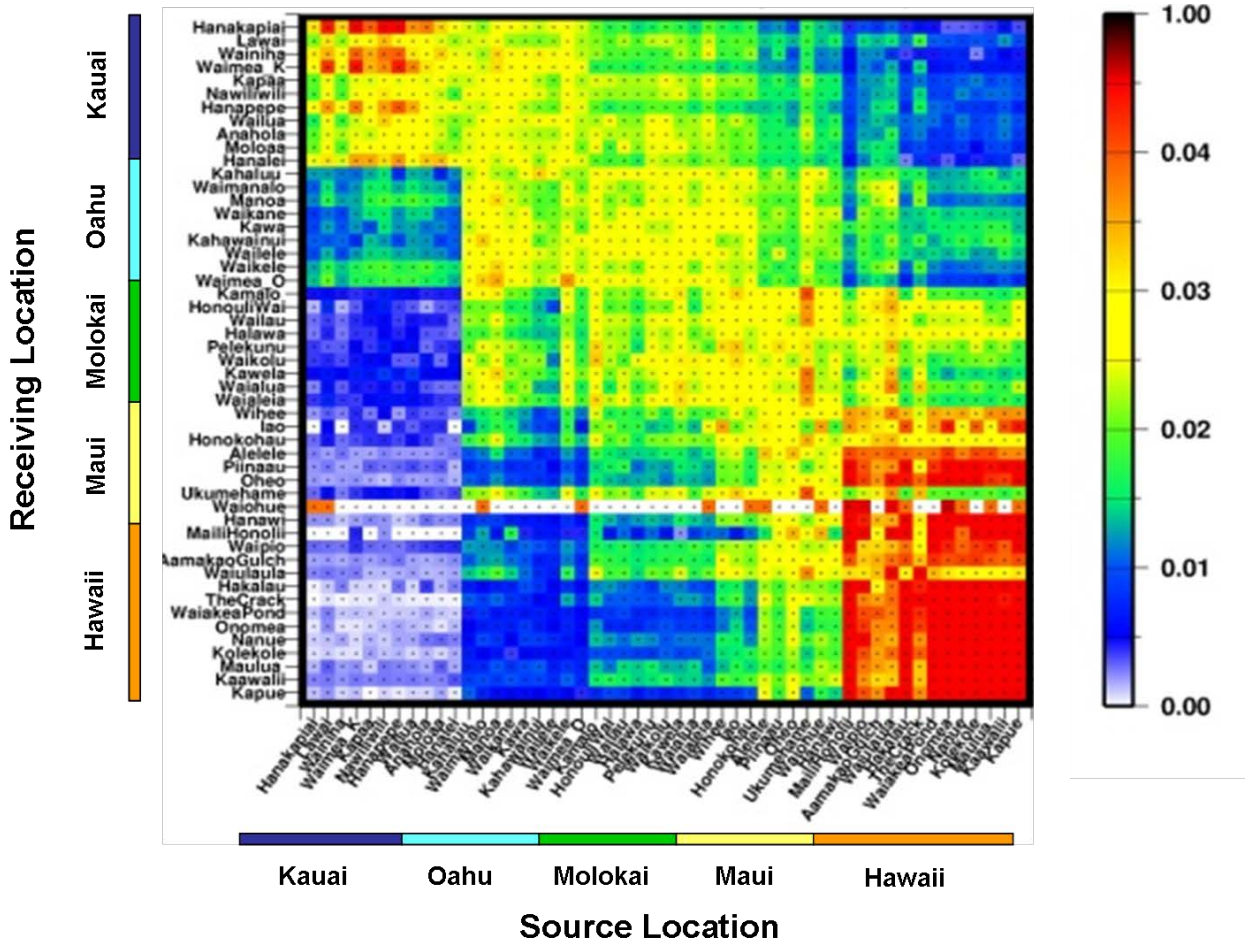

Figure 31: Connectivity matrix showing settlement probabilities for 51 streams on five islands in the main Hawaiian Islands for a representative 55 day LLD run with constant spawning output between locations and through time. Kauai watersheds are on top of the y-axis and to the far left of the x-axis; watersheds on the island of Hawaii are on the bottom of the y-axis and to the far right of the x-axis. Self-recruitment is represented along the diagonal of the probability matrix.

Hawaii and Kauai occur under both short and long LLD scenarios. The proportion of larvae that are lost at sea is much higher under short LLD conditions, with most of the lost larvae originating on Kauai (Figures 31 and 32). However, there is also greater self-recruitment on Kauai (and other islands) under conditions of short LLD, and a general trend of isolation-by-distance, where the likelihood of dispersal among watersheds decreases with increasing intervening distances. Under conditions of longer LLD, larvae generally disperse greater distances, increasing connectivity between the southeastern and northwestern regions of the main Hawaiian Islands.

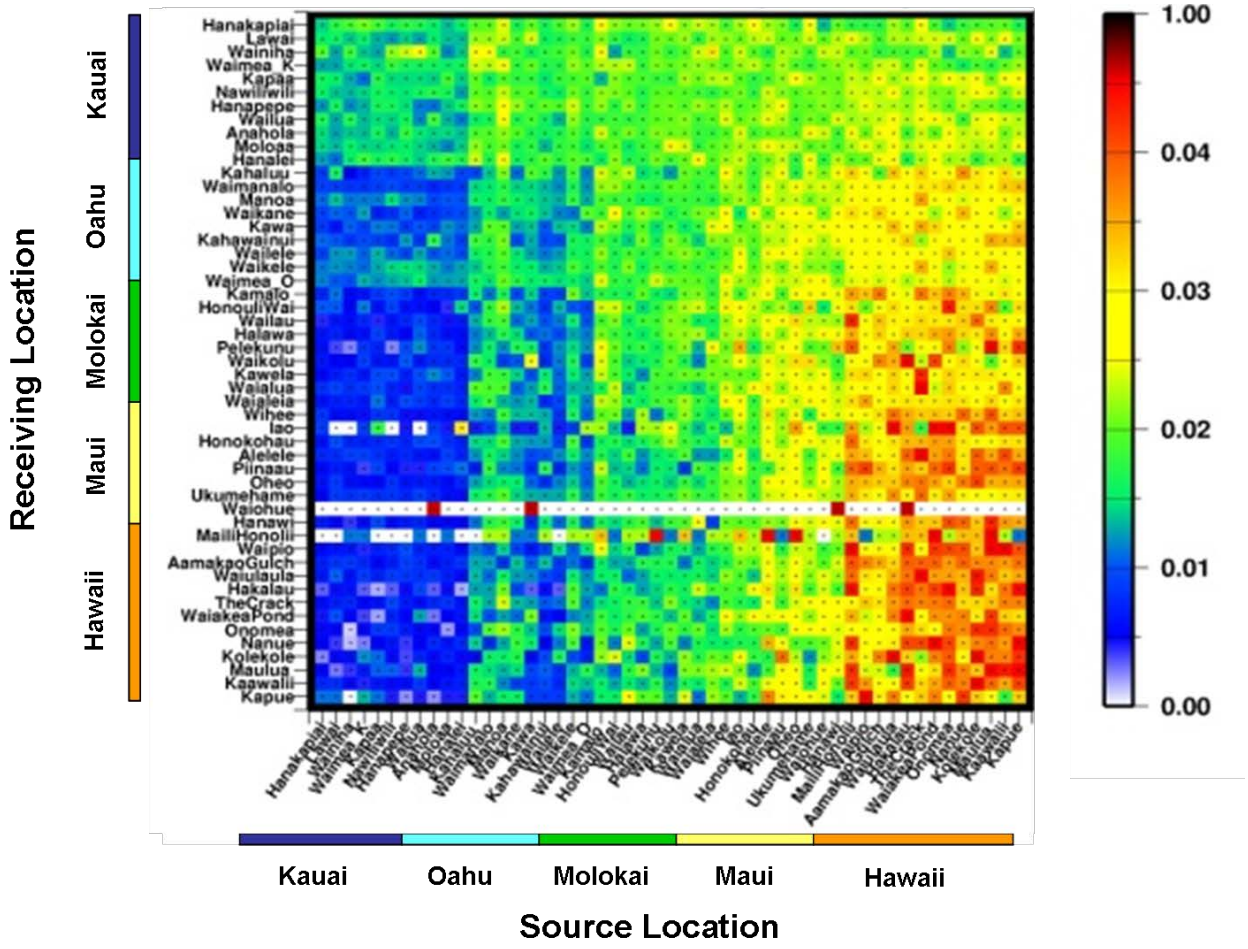

Figure 32: Connectivity matrix showing settlement probabilities for 51 streams on five islands in the main Hawaiian Islands for a representative 150 day LLD run with constant spawning output between locations and through time. Kauai watersheds are on top of the y-axis and to the far left of the x-axis; watersheds on the island of Hawaii are on the bottom of the y-axis and to the far right of the x-axis. Self-recruitment is represented along the diagonal of the probability matrix.

***Dispersal modeling with variable larval export*:** In comparison to model outcomes under conditions of constant larval export, propagule pressure originating from the island of Hawaii contributed less to the larval pools capable of reaching watersheds on other islands in the archipelago (Figure 33). Self-recruitment on Hawaii and on other islands also was markedly higher under conditions of variable larval export. Dispersal remained asymmetrically weighted

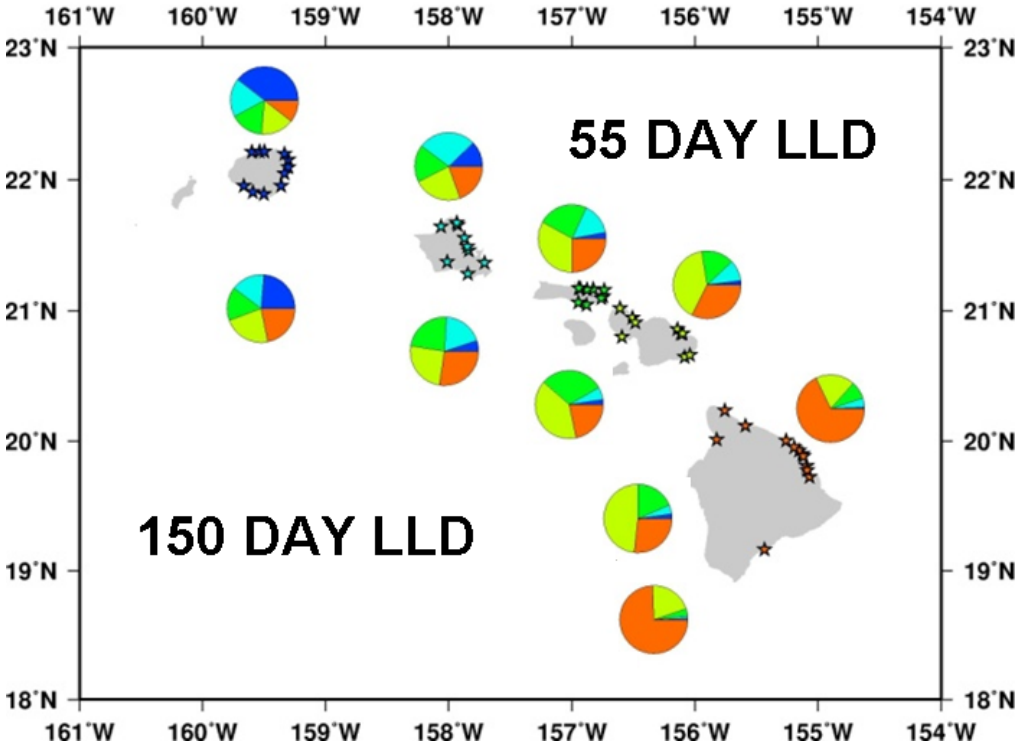

Figure 33: The proportion of successfully settled propagules according to their island of origin for Kauai (dark blue), Oahu (light blue), Molokai (green), Maui (yellow) and Hawaii (orange) under conditions of short larval life duration (55 days), and long larval life duration (150 days). Spawning output was variable between locations and through time. Stars indicate the location of stream mouths for watersheds where larval propagules entered the oceanic environment.

from east to west under variable export conditions (Figure 33). Larvae originating from Kauai did not reach Hawaii in 55 LLD runs, though a small fraction of larvae reached Hawaii under conditions of longer LLD (Figure 33).

The probability matrices illustrate that connectivity among watersheds is weaker under variable larval export (Figures 34 and 35). The matrices also illustrate an east-to-west dispersal asymmetry among watersheds, with connectivity among watersheds being weakest among the far eastern and far western regions of the archipelago (Figures 34 and 35). This likely reflects the direction of prevailing currents, the distribution of the focal watersheds, and higher probabilities of self-recruitment across the archipelago. However, there are a few exceptions to the dominant trends. For example, under constant spawning conditions, low connectivity was found for Waiohue watershed with other streams on Maui, whereas high connectivity was found for the watershed under the variable stream flow scenario (Figures 34 and 35).

Discussion: The simulation results of the HYCOM-based, coupled biophysical model indicate that, under basic conditions of advection and diffusion, larval dispersal is restricted and asymmetric across the archipelago. These findings are highly conservative because the model simulations do not account for additional factors that could further constrain propagule pressure and movement potential. Dispersal could be further constrained by variation in propagule pressure arising from differences in adult densities among watersheds. For example, propagule pressure is likely depressed by low adult densities in watersheds across Oahu. Based on the

outcomes of runs assuming larval input scales to flow regime, this could reduce self-recruitment and total recruitment across the island. If so, then negative feedback cycles could emerge that might precipitate extirpation of adult populations on the island. The model simulations also do not account for evidence from otoliths of life history variation, as has been observed among *A. stamineus*, where the majority of larvae either remain in fresh water or occupy near-shore habitats. Facultative amphidromy would increase the proportion of self-recruitment by reducing the pool of larvae dispersing among watersheds and islands. Occupancy of near-shore habitats might also increase self-recruitment or possibly reduce dispersal distances (i.e., to proximate watersheds or proximate islands).

The model outcomes are broadly consistent with dispersal patterns inferred from analyses of genetic variation. Analyses of microsatellite variation in *Sicyopterus stimpsoni* and *Awaous stamineus* suggest that gene flow (and by extension, dispersal) is high among populations across

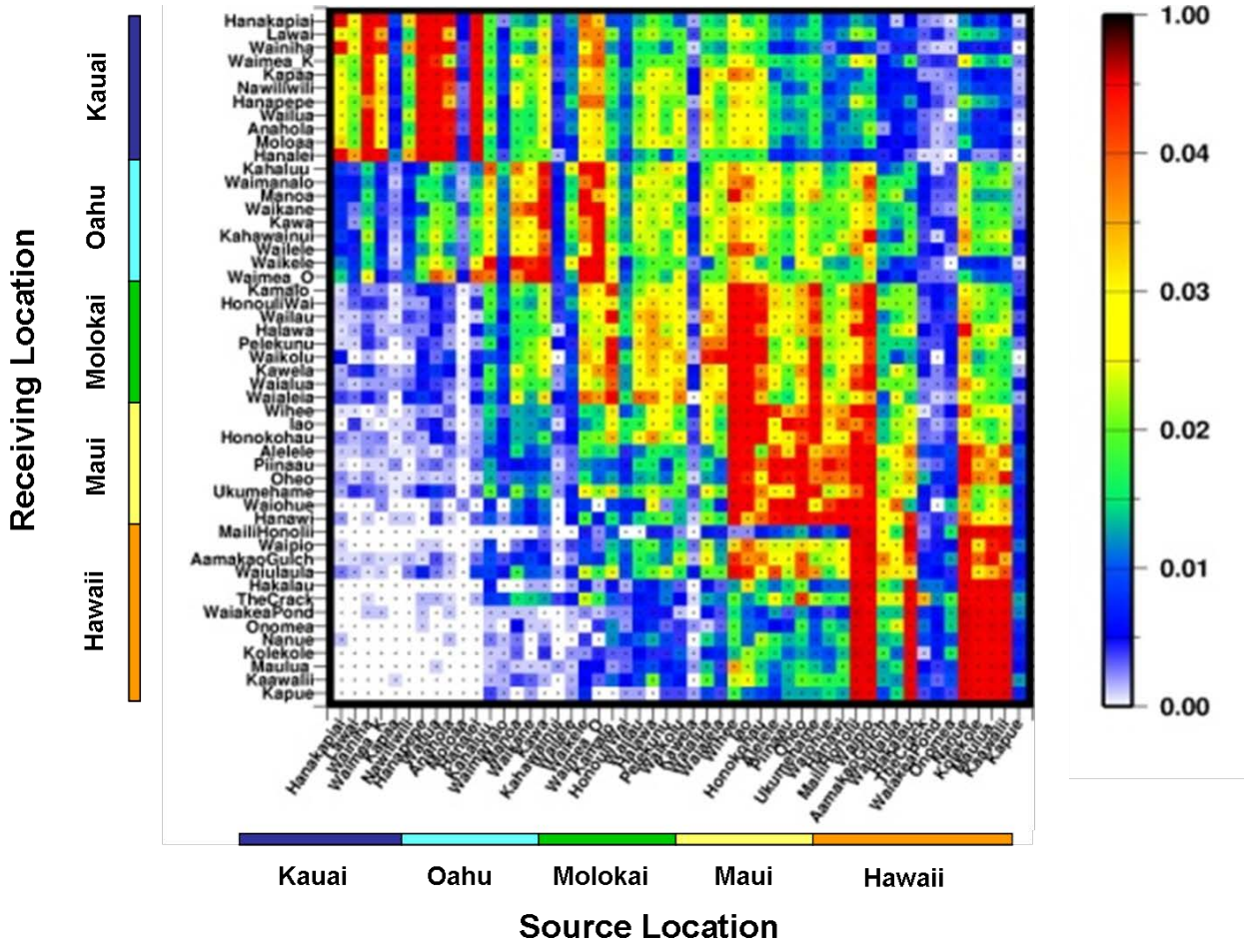

Figure 34: Connectivity matrix showing settlement probabilities for 51 streams on five islands in the main Hawaiian Islands for a representative 55 day LLD run with variable spawning output between locations and through time. Kauai watersheds are on top of the y-axis and to the far left of the x-axis; watersheds on the island of Hawaii are on the bottom of the y-axis and to the far right of the x-axis. Self-recruitment is represented along the diagonal of the probability matrix.

the archipelago, but that it is stochastic. Both HWE and measurements of population differentiation (F_{ST} and AMOVA) in *S. stimpsoni* vary between years, streams, and age classes (i.e., juveniles versus adults). Patterns of allelic richness in *S. stimpsoni* also are indicative of differences in the contribution of source populations between years, where allelic richness was greater among adult subpopulations compared to juvenile cohorts when juveniles were subdivided by year, but not when juvenile cohorts were combined across years within a watershed (K. Moody, unpublished data). This pattern could emerge because of differences in reproductive output among adult subpopulations over time, which could yield sweepstakes recruitment (Hedgecock 1994, Larson and Julian 1999). It may also be attributable to differences in ocean currents and eddies over time, which could reduce admixture of larval pools and result in stochastic or irregular larval transport (Zardi et al. 2011). Model simulations suggest that unstable or shifts in oceanic circulation alone could give rise to the observed genetic patterns. However, additional simulations that incorporate differences among adult population densities will be required to gauge the relative influence of physical parameters and variable reproductive potential on dispersal.

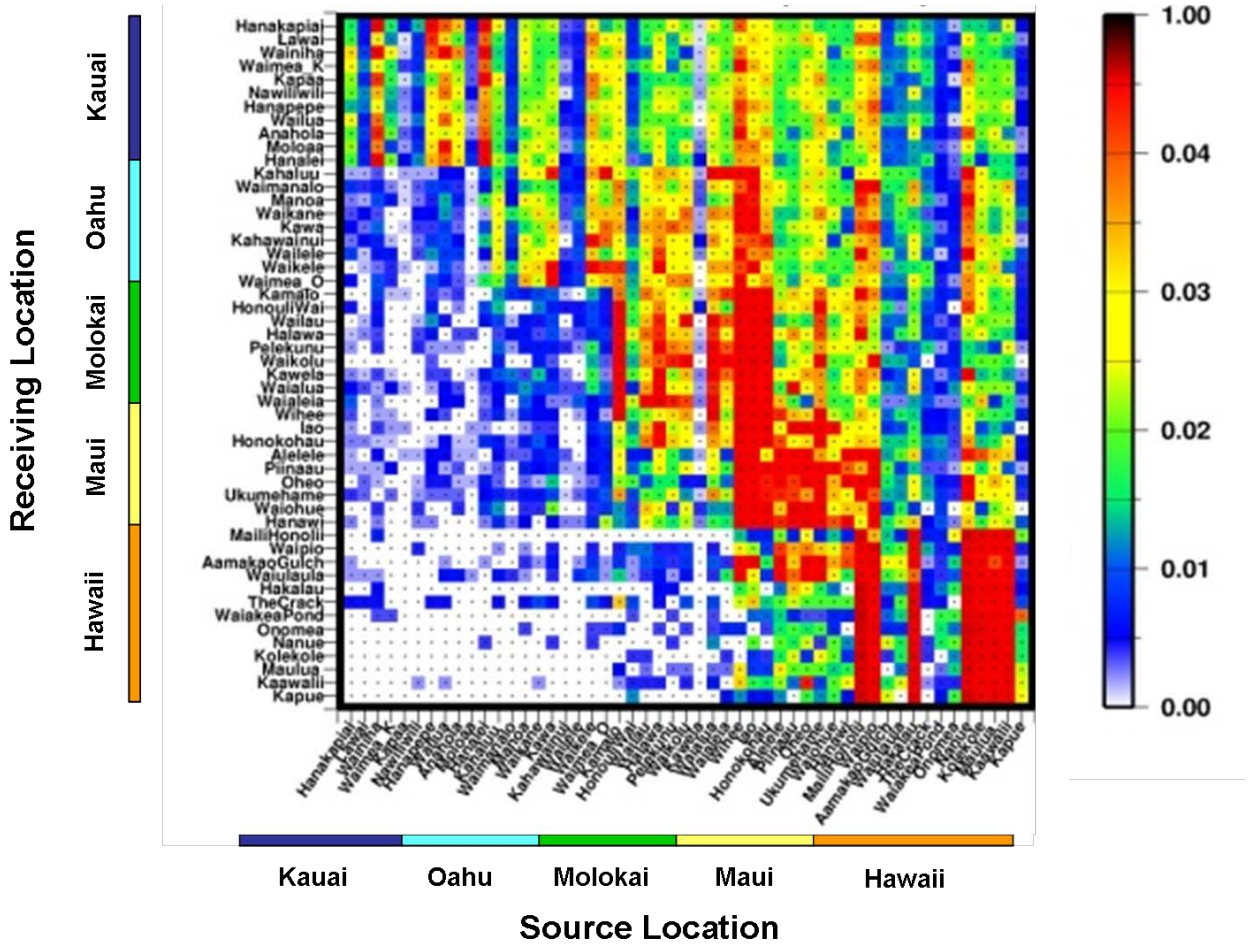

Figure 35: Connectivity matrix showing settlement probabilities for 51 streams on five islands in the main Hawaiian Islands for a representative 150 day LLD run with variable spawning output between locations and through time. Kauai watersheds are on top of the y-axis and to the far left of the x-axis; watersheds on the island of Hawaii are on the bottom of the y-axis and to the far right of the x-axis. Self-recruitment is represented along the diagonal of the probability matrix.

Differences in genetic variation among *Sicyopterus stimpsoni* and *Awaous stamineus* indicate that conditions capable of mitigating the influence of marine transport on dispersal likely vary among species. For example, both species exhibited evidence of population differentiation, but only *A. stamineus* exhibited a strong pattern of isolation-by-distance where population differentiation increased with greater intervening geographic distances. In contrast, *S. stimpsoni* exhibited more stochastic patterns of population differentiation. This suggests that species-specific attributes (i.e., behaviors or life history parameters) give rise to variation in dispersal independently of prevailing patterns of ocean circulation. Additional model simulations that account for documented or inferred behavioral and life history variation would clarify how movement potential can differ among co-occurring species. Similarly, additional model simulations that account for differences in adult population densities among species could result in more accurate characterization of connectivity and serve as a tool for testing hypotheses of source-sink dynamics.

The discovery of life history variation in *A. stamineus*, where individuals exhibit signatures of facultative amphidromy and local retention, suggests that models of advection-diffusion dynamics are too simplistic, and that greater consideration should be given to near-shore currents. Loss of amphidromy can evolve and persist because it can advantageously ensure recruitment to (or replenishment of) resident populations. However, larval dispersal through the marine environment is associated with enhanced larval condition and adult condition, which can translate in to increased lifetime fitness (Hogan et al., in review). Accordingly, the life history and LLD polymorphisms observed in *A. stamineus* may reflect a balance between the costs and benefits of marine dispersal. Both in-stream conditions and local retention by near-shore currents and eddies are possible contributing factors to (or stepping stones toward) the evolution or expression of non-migratory phenotypes (Michel et al. 2008). Evidence from otolith microchemistry and model simulations illustrate the importance of local recruitment and retention in *A. stamineus* population connectivity. Further modeling of near-shore conditions, and accounting for the possibility of active larval retention (i.e., via vertical migrations), could illustrate whether there are causative or correlative relationships between circulation patterns and life history variation. Amending the model to reflect a more continuous distribution of LLDs (i.e., as revealed by otolith microchemistry analyses) might also shed additional light on the nature of these relationships. Experimental studies involving modification of in-stream conditions could also illustrate whether non-migratory phenotypes are a function of phenotypic plasticity or heritable variation.

Though *S. stimpsoni* did not exhibit a genetic pattern of isolation-by-distance, a strong pattern of isolation-by-environment was found in a morphological analysis of newly recruiting juveniles and resident adult populations (K. Moody, unpublished data). Body shape and morphological traits exhibited by the two age classes were found to be more similar within environmental types (e.g., steep stream slope gradient versus low stream slope gradient) than between environmental types. This suggests that either stream-specific post-settlement selective pressures or selection acting on larvae at sea (and thereby producing genetically distinct recruitment events) are reducing population connectivity, and that neither neutral genetic markers nor the HYCOM-based model are capturing this process. If stream-specific selection is the cause, the pattern of

heterogeneity would remain constant through time. On the other hand, if at-sea selection is the cause, the locations of genetically distinct populations would likely change. Time-series allele frequency data are needed to investigate the relative importance of stream-specific or at-sea selection.

Coupling the HYCOM-based biophysical model with individual-based models (IBMs) that encompass in-stream biotic and abiotic selective pressures would likely yield a better understanding of population connectivity within and among islands. Outputs (ie. immigration rates) from the biophysical model could support an IBM stream model designed to follow the fate of individual larvae once they have arrived in the nearshore waters of each island and watershed. Each island or watershed could be modeled as a separate spatially-explicit individual-based population parameterized for stream flow rates, productivity, predation risk, and topographical complexity. Outputs from these models could then provide the basis for establishing the number of larvae that would be redistributed (or retained, or entrained) according to probability distributions generated by the HYCOM-based biophysical model.

Comparisons of model simulations to inferences drawn from other sources of information (i.e., genetic variation, otolith microchemistry, morphological variation) illustrate that dispersal is a dynamic process that must be considered from both spatial and temporal perspectives. Conclusions drawn from a single location (i.e., multiple cohorts sampled at one location), a single point in time (i.e., single cohorts sampled from multiple locations), or a single age class, may result in misleading information about population connectivity and movement potential. In the absence of additional information, it is possible that erroneous conclusions may be drawn about the relative importance of stochastic (e.g., irregular currents) versus deterministic (e.g., directional currents) conditions. Without broadly encompassing information on spatial and temporal variability, it can also prove difficult to differentiate between potential evolutionary (e.g., post-settlement selection) and ecological drivers (e.g., local entrainment by near-shore currents, differential reproductive output) of dispersal (Larson and Julian 1999, Limborg et al. 2012). Simulating spatial and temporal variability and comparing modeling results to estimates of dispersal derived from independent approaches provided further evidence that larval pool admixture is not as prevalent as has been previously proposed (Selkoe et al. 2006; Siegel et al. 2008; Pringle et al. 2009). It also provided further evidence that the potential for long distance dispersal does not always accurately predict realized dispersal distance (Shanks et al. 2009; Weersing and Toonen 2009).

5.1 Genetic and Integrative Assessment of Pacific Island Watersheds

5.1.1 Among-Watershed Assessment of Environmental Variation

Watershed land use: The first two principal components for land use across watersheds and islands corresponded to urbanization and canopy cover, respectively. The first PC factor (PC1) had negative loadings of natural land uses (forests, canopy, and wetlands), and positive loadings of anthropogenic land uses (development, agriculture, and impervious surface). The second PC

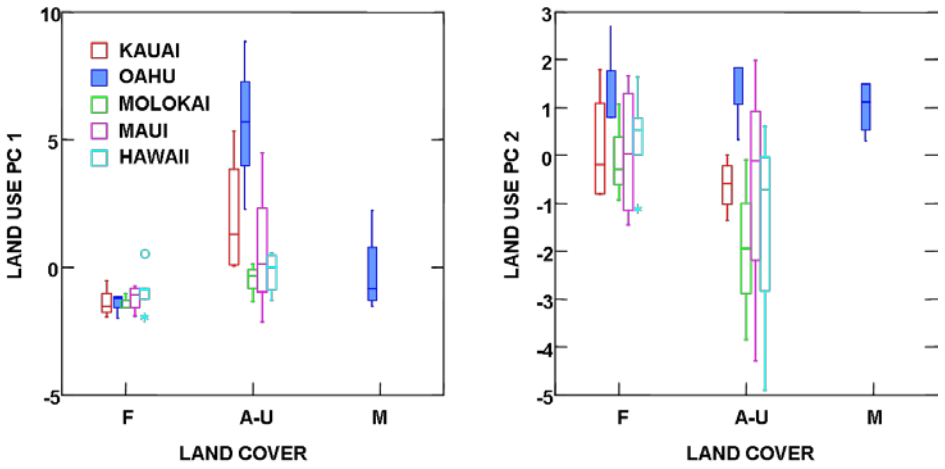

Figure 36: Comparison of land use PC1 (left) and land use PC2 (right) among watersheds across islands, with specific reference to Oahu, according to dominant land cover (F = forest, A-U = ag-urban) and stewardship (M = military). ANOVA values are provided in the text.

factor (PC2) corresponded to a gradient from low canopy land uses (grasslands and shrubs) to high canopy land uses (i.e., forests). Even though PC1 was significantly correlated with %ag-urb and %forest ($p < 0.05$), which we selected *a priori* to serve as land use metrics, both PC1 and PC2 were retained for subsequent pair-wise comparisons to in-stream conditions and to evaluate biotic responses to land use gradients.

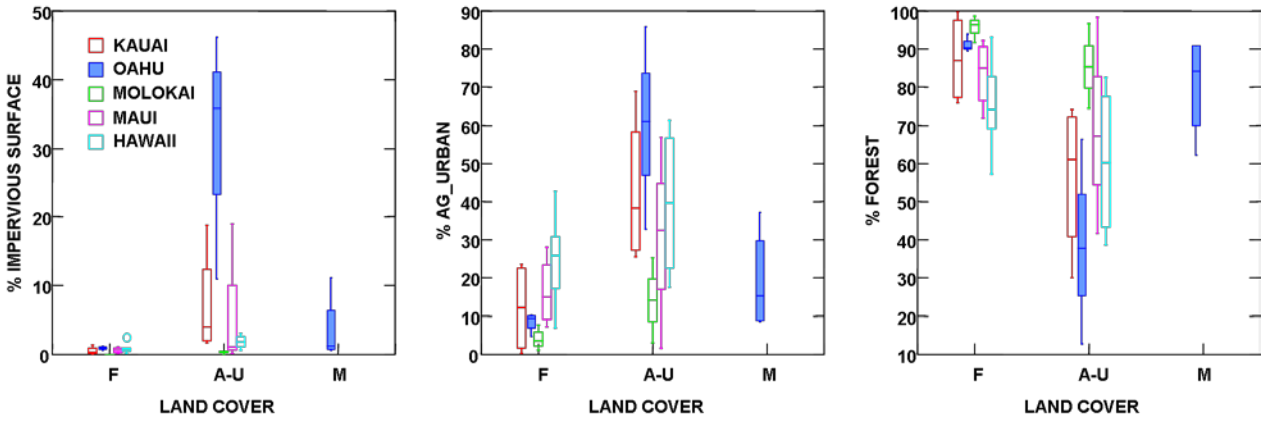

Figure 37: Comparison of % impervious surface (left), %ag-urban (middle) and %forest (right) land cover among watersheds across islands, with specific reference to Oahu, according to dominant land cover category (F = forest, A-U = ag-urban) and stewardship (M = military). ANOVA values are provided in text.

Islands and watershed land use categories exhibited differences in PC1 values (ANOVA, all $p < 0.05$) and in values of constituent variables like the percentage of impervious surfaces and %ag-urb (ANOVA, all $p < 0.05$). Values of PC2, though generally higher in forested watersheds, did not differ among islands or by watershed land use category (ANOVA, all $p > 0.05$). Comparisons also showed that many of the highest PC1 values and lowest %forest were recovered in ag-urban watersheds on Oahu (Figures 36 and 37).

Water chemistry: There was a positive relationship between water chemistry PC1 (N, solutes, and solids) and land use PC1 (i.e., intensification) in 2009 ($r^2 = 0.55$, $p < 0.001$) and 2011 ($r^2 = 0.26$, $p < 0.001$; Figure 38). The weaker relationship recovered for 2011 is likely attributable to fewer sites being sampled in heavily urbanized areas. A significant relationship was not

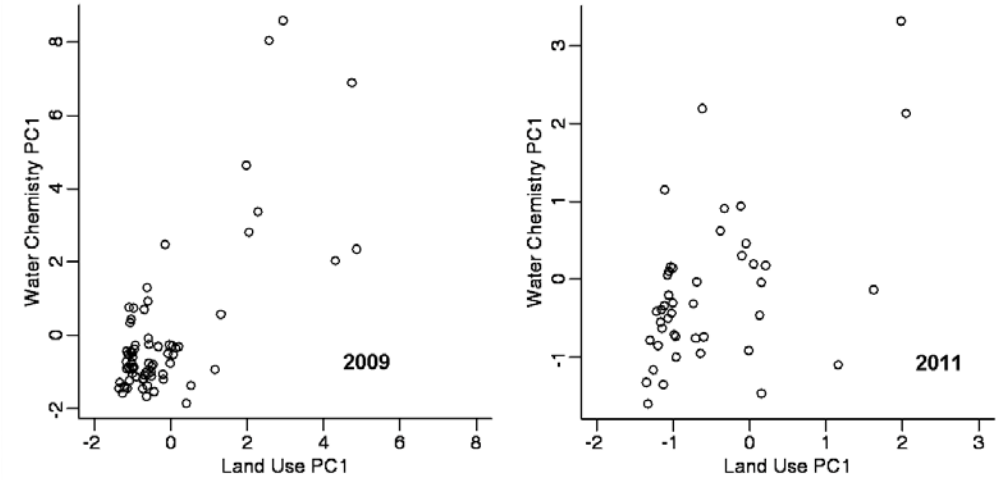

Figure 38: Comparison of watershed chemistry PC 1 to land use PC1 (i.e., intensification) among watersheds in 2009 (left) and 2011 (right). Correlation values are provided in text.

recovered between water chemistry PC1 and land use PC2 ($p > 0.05$). No significant relationships were recovered between SRP and either of the land use principal components ($p > 0.05$). For nearly all of the individual water chemistry parameters measured, values obtained in 2009 were significantly correlated with measurements made at the same locations in 2011 at the site scale (site, $0.312 < r < 0.962$, $p < 0.05$). The exceptions were temperature, pH, TSS, and TP (site, $0.06 < r < 0.227$, $p > 0.05$). Parallel relationships were recovered at the watershed scale, except weaker relationships were recovered for TDS ($r = 0.344$, $p = 0.228$) and NH4 ($r = 0.09$, $p = 0.76$), and a stronger relationship was recovered for TSS ($r = 0.611$, $p = 0.02$). PC1 values obtained for 2009 were highly correlated with those obtained for 2011 at both site and watershed scales (site, $r = 0.957$, $p < 0.001$; watershed, $r = 0.728$, $p = 0.003$; Figure 39). PC2 values were significantly correlated only at the watershed scale (site, $r = 0.13$, $p = 0.718$; watershed, $r = 0.675$, $p = 0.008$; Figure 39). Though many water chemistry parameters do not reflect time-integrated conditions (i.e., the parameters reflect conditions that can be highly dynamic over time and space), this suggests that water chemistry conditions at the study sites were generally consistent across the study period.

Categorical comparisons of water chemistry conditions among islands by dominant land use indicated that forested watersheds generally exhibit lower water chemistry PC1 values (Figure 40), though differences among islands or by land use were not statistically significant (ANOVA, $p > 0.05$). Comparisons nonetheless indicate that many of the highest PC1 values were recovered in ag-urban watersheds on Kauai and in areas under military stewardship on Oahu (Figure 40). Similarly, no significant differences were found among water chemistry PC2 values by island or land use category (ANOVA, $p > 0.05$), though the highest values were recovered for forested watersheds on Molokai (Figure 40).

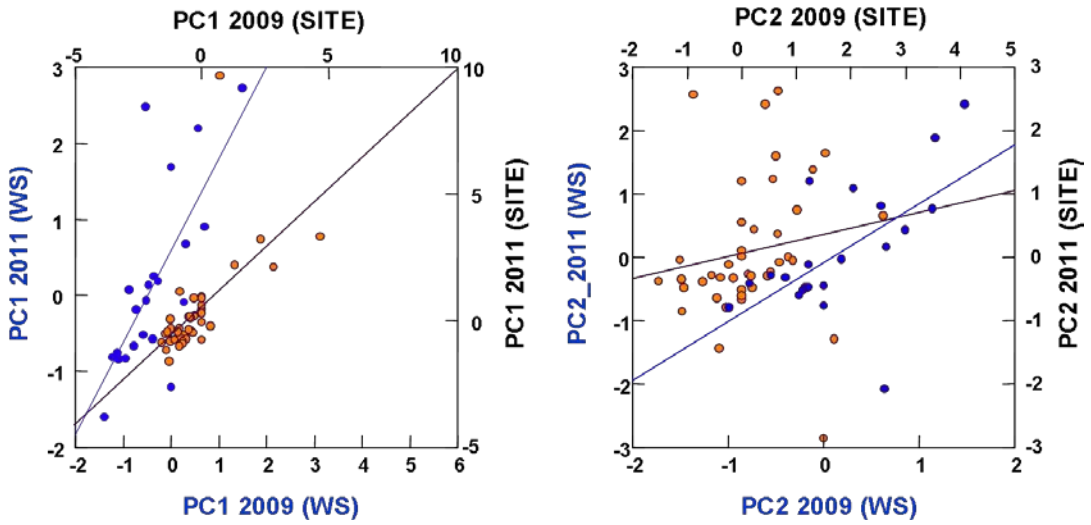

Figure 39: Comparison of water chemistry PC1 and PC2 values between 2009 and 2011 for both site (orange and black) and watershed (blue) spatial scales. Correlation values are provided in the text.

Stable isotopes: A strong positive relationship was recovered between 2009 water chemistry PC1 and *A. stamineus* $\delta^{15}N$ values ($r^2 = 0.70$, $p < 0.001$; Figure 41); this relationship was weaker but still significant for the 2011 data ($r^2 = 0.38$, $p < 0.001$). Both algae and snail $\delta^{15}N$ values had similar relationships with water chemistry PC1 in 2009 (algae: $r^2 = 0.57$, $p < 0.001$; snails: $r^2 = 0.54$, $p < 0.001$) and in 2011 (algae: $r^2 = 0.29$, $p < 0.001$; snails: $r^2 = 0.32$, $p < 0.001$). The reduction in the strength of the relationships between stable isotope values and water chemistry PC1 is likely due to the narrower range of PC1 values and weaker differentiation between SRP and the other water chemistry variables in the 2011 data set.

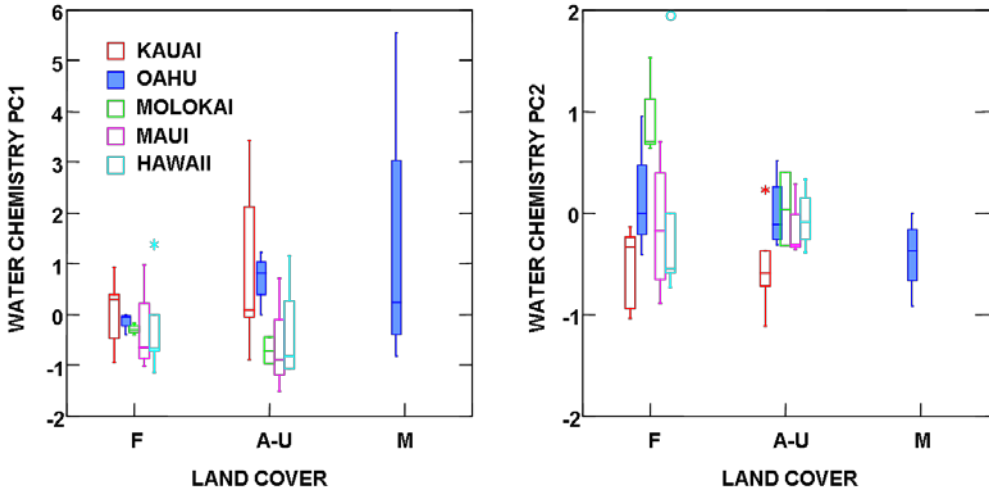

Figure 40: Comparison of water chemistry PC1 and PC2 values averaged over 2009 and 2011 among watersheds across islands, with specific reference to Oahu, according to dominant land cover (F = forest, A-U = ag-urban) and stewardship (M = military). ANOVA values are provided in the text.

Like the observed trends in water chemistry, categorical comparisons of stable isotope values among islands by dominant land use indicated that forested watersheds generally exhibit lower $\delta^{15}N$ values (Figure 42), though differences among islands or by land use were not statistically significant (ANOVA, $p > 0.05$). Comparisons nonetheless show that many of the highest $\delta^{15}N$ values were recovered in ag-urban watersheds and in areas under military stewardship on Oahu (Figure 42).

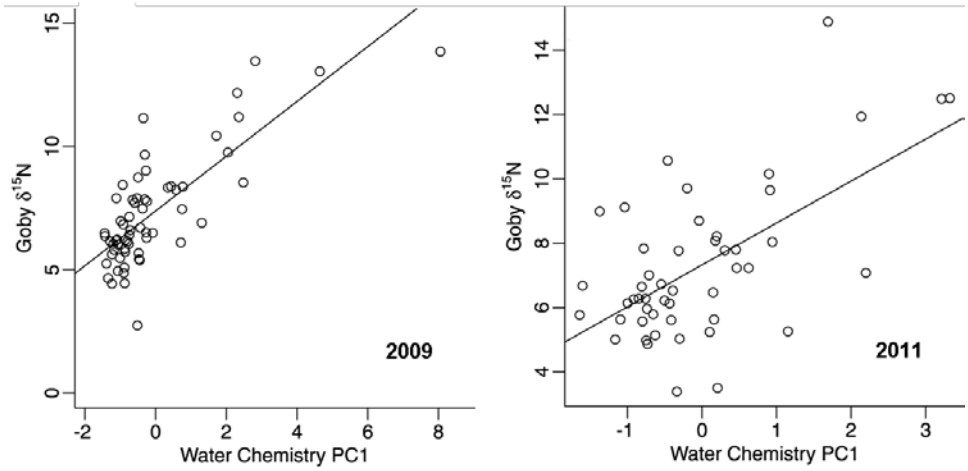

Figure 41: Comparison of watershed chemistry PC1 to goby $\delta^{15}N$ among watersheds in 2009 (left) and 2011 (right). Correlation values are provided in the text.

Awaous stamineus inhabiting sites with higher water chemistry PC1 scores occupied higher trophic positions (i.e., individuals had more animal matter in their diet; Figure 43). Linear regression after log-transformation of water chemistry PC1 yielded a significant positive relationship between water quality and trophic position for 2009 ($r^2 = 0.36$, $p < 0.001$; Figure 43). Neither trophic position nor *A. stamineus* $\delta^{15}N$ were related to *A. stamineus* size, indicating that the observed shift is not attributable to ontogenetic shifts in diet. Trophic position of *A. stamineus* was fairly consistent around 2.5 when *Neritina granosa* or *Sicyopterus stimpsoni* was present.

***Discussion*:** We have identified a diagnostic set of water chemistry conditions associated with urban development in the Hawaiian archipelago. Increases in sediments, solutes, and nitrogen all appear to be linked to urban development. Development in continental watersheds also typically yields higher P levels (Johnes 1996, Carpenter et al. 1998, Walsh et al. 2005), but we found no evidence of a relationship between urbanization and P concentrations in the Hawaiian Islands. We also found that *A. stamineus* occupy higher trophic positions at more urbanized sites, which indicates that urbanization changes resource availability for native species in Hawaiian Island watersheds.

The observed covariance among a suite of water chemistry variables, with the exception of P, suggests that urbanization is a common driver of surface water quality conditions. Urban development described half of the observed variation in water chemistry, indicating that stormwater runoff, sewage inputs, and other urban influences play a large role in determining

water quality in Hawaiian Island streams. The consistency of patterns between years indicates that the influence of urbanization on stream conditions is relatively stable and is not driven by transient events, such as spates (i.e., flooding) and other short-term events.

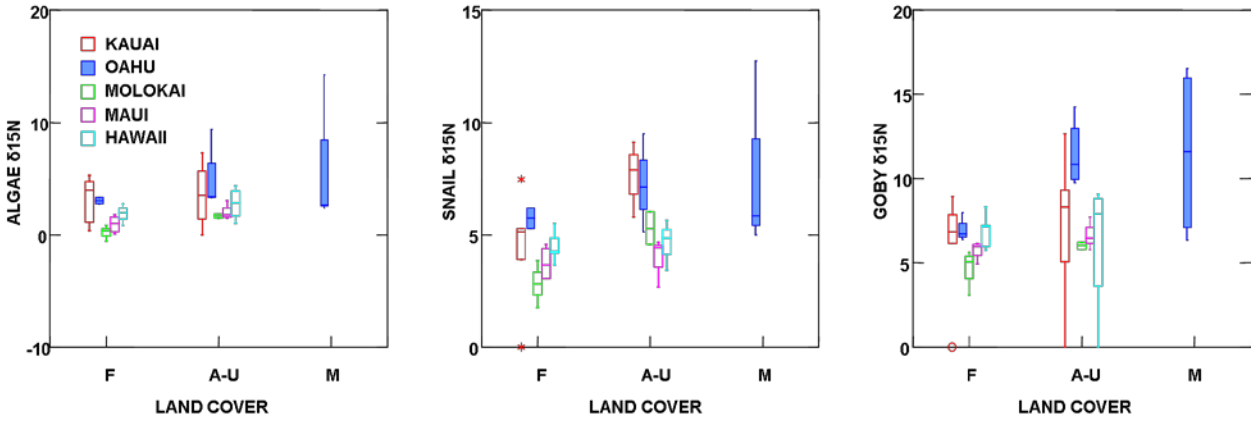

Figure 42: Comparison of $\delta^{15}N$ values for algae (left), snails (middle) and native gobies (right) averaged over 2009 and 2011 among watersheds across islands, with specific reference to Oahu, according to dominant land cover (F = forest, A-U = ag-urban) and stewardship (M = military). ANOVA values are provided in the text.

In continental watersheds, urban and agricultural development typically yields elevated levels of nitrogen and phosphorus, but no relationship between phosphorus and land cover was found for Hawaiian Island streams. Weathering of rocks is the primary source for phosphorus in natural ecosystems (Smil 2000), and the strong age gradient across the Hawaiian Islands generates large differences in phosphorus availability (Crews et al. 1995). Young and old soils have lower phosphorus availability than intermediate aged soils. Though variation in phosphorus is likely attributable to weathering gradients across the archipelago, further work will be required to identify the primary controls of phosphorus in Hawaiian streams.

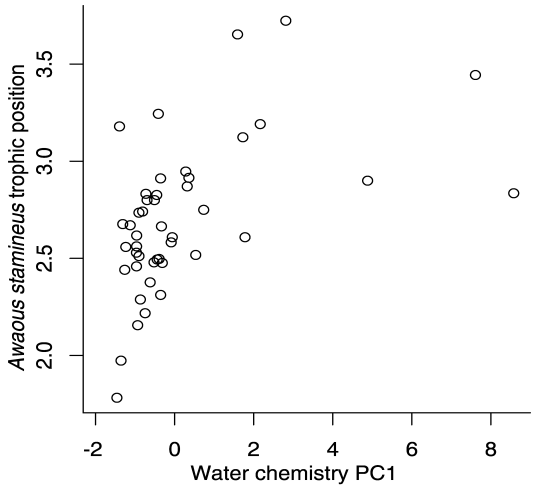

Figure 43: Comparison of watershed chemistry PC1 to *Awaous stamineus* trophic position among watersheds in 2009. Correlation values are provided in the text.

Stable isotopes are increasingly being used as indicators of anthropogenic nitrogen contributions to aquatic ecosystems (Anderson and Cabana 2006, Anderson and Cabana 2007, Bergfur et al. 2009, Diebel and Vander Zanden 2009), including coastal wetlands in Hawaii (Bruland and MacKenzie 2010). The strong relationship between water chemistry PC1 and $\delta^{15}N$ values of gobies also links human activity to in-stream conditions. Anthropogenic nitrogen inputs tend to increase nitrogen isotopic ratios. Some anthropogenic sources are enriched in ^{15}N (e.g., wastewater and animal manure). Though atmospherically derived fertilizers tend to have isotopic ratios around zero, cycling by soil microbes and denitrification can result in elevated isotopic ratios (Heaton 1986). The tight relationship between water quality and consumer ^{15}N indicates that

nitrogen isotopes of widespread consumer species can serve as a useful tool for monitoring stream conditions in Hawaii. Though the observed differences were not statistically significant, cross-island comparisons uncovered trends that indicate that water quality conditions on Oahu and Kauai are more impaired than on other islands, and that watersheds under military stewardship exhibit some of the highest levels of nutrient loading in the archipelago.

The observed shifts of *A. stamineus* to higher trophic positions with in-stream degradation indicate that resource availability and community organization change with water quality. Similar outcomes have been found in continental stream ecosystems, where the presence and relative abundance of different functional feeding groups can change with in-stream conditions (Poff et al. 2006). The shifts in trophic position indicate that *A. stamineus*, which is considered to be an omnivorous species, has a labile diet. When *A. stamineus* is sympatric with endemic grazers, such as *N. granosa* or *S. stimpsoni*, the species occupies a uniformly low trophic position, suggesting that algal resource availability influences its diet. By smothering algae, fine sediments in urban streams can reduce the availability of autochthonous basal resources (Brasher 2003), which could drive *A. stamineus* towards alternative food resources and a higher trophic position. Alternatively, the shift in trophic position may reflect changes in prey availability, given that urban development also influences the benthic invertebrate community in Hawaiian streams (Brasher 2003, Englund et al. 2007).

5.1.2 Sensitivity of Genetic, Population, & Community Metrics to Among-Watershed Environmental Variation

Genetic diversity in Awaous: In 2009, *Awaous stamineus* were sampled at 91 sites in 39 watersheds, with sample sizes per watershed ranging from 1 to 69 individuals, inclusive of both adults and postlarvae (Table 1). In 2011, *A. stamineus* were sampled at 80 sites in 33 watersheds, with sample sizes per watershed ranging from 4 to 70 individuals inclusive of both adults and postlarvae (Table 1). Over both years, samples were obtained at a total of 35 sites in 12 watersheds on Oahu, including five watersheds supporting military installations (e.g., Waimanalo watershed) or activity (e.g., Waimea watershed). Genotype and/or mtDNA sequence data were obtained for 1103 individuals in 2009 and 1410 individuals in 2011. A total of 49 mtDNA haplotypes were recovered from 1035 individuals sampled in 2009. A total of 249 alleles and an average of 19.15 alleles per locus were recovered from the genotyped individuals in 2009. A total of 83 mtDNA haplotypes were recovered from 1219 specimens sampled in 2011, with 52 of those haplotypes being distinct from those recovered in 2009. A total of 261 alleles and an average of 18.6 alleles per locus were recovered from individuals sampled in 2011.

The range of variation observed at measures of genetic diversity was similar for locations sampled in 2009 and 2011. For example, expected heterozygosity varied from 0.46 to 1 and from 0.59 to 0.76 among all watersheds in 2009 and 2011, respectively. Expected heterozygosity varied from 0.42 to 1 and 0.50 to 0.85 among all sites in 2009 and 2011. Rarified allelic richness ranged from 1.46 to 2 and from 1.58 to 1.76 among all watersheds in 2009 and 2011. Allelic richness varied from 1.43 to 2 and 1.50 to 1.85 among all sites in 2009 and 2011. Shannon diversity of alleles varied from 0.32 to 1.67 and the average number of alleles per locus ranged from 1.46 to 11.23 among all watersheds in 2009. Shannon diversity varied from 1.20 to 1.73 and the average number of alleles per locus ranged from 4.23 to 11.08 among all watersheds in

2011. Shannon diversity varied from 0.30 to 1.72 and 0.35 to 1.64 among all sites sampled in 2009 and 2011, respectively. The average number of alleles per locus ranged from 1.43 to 10.15 and 1.5 to 10.14 among all sites sampled in 2011, respectively. In a given year, mitochondrial haplotype diversity varied between 1 and 15 per watershed, with cumulative values ranging as high as 20 per watershed.

Though levels of genetic diversity in *Awaous stamineus* varied among sites and watersheds (see below), no significant differences were found among islands (Figure 44). In 2009, Shannon diversity was slightly lower on Oahu, but post-hoc pairwise Tukey tests showed that differences among islands were not statistically significant (watersheds ANOVA, $p = 0.493$; pair-wise comparisons, $p > 0.431$; sites ANOVA, $p = 0.461$; pair-wise comparisons, $p > 0.56$). Similarly, the average number of alleles per locus (NAA) was lowest on Oahu and greatest on Molokai, but the differences were not statically significant (watersheds ANOVA, $p = 0.741$; pair-wise comparisons, $p > 0.713$; sites ANOVA, $p = 0.750$; pair-wise comparisons, $p > 0.813$). The lowest average number of mtDNA haplotypes was recovered on Hawaii than other islands, though the difference in haplotype diversity among islands was not statistically significant (watersheds ANOVA, $p = 0.150$; pair-wise comparisons, $p > 0.138$; sites ANOVA, $p = 0.313$; pair-wise comparisons, $p > 0.407$). Parallel findings were recovered for sites and watersheds sampled in 2011, and for values averaged across years.

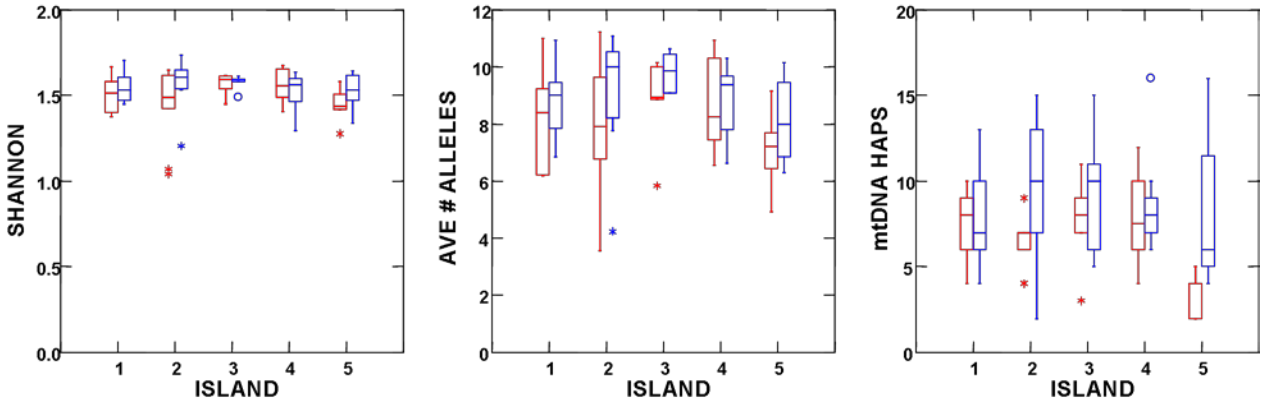

Figure 44: Comparison of watershed-scale estimates of *Awaous stamineus* nuclear microsatellite allelic diversity (Shannon = Shannon diversity, Ave # alleles = average number of alleles per locus) and mitochondrial haplotype diversity (mtDNA haps = number of mtDNA haplotypes) in 2009 (red) and 2011 (blue) across islands (1 = Kauai, 2 = Oahu, 3 = Molokai, 4 = Maui, 5 = Hawaii). ANOVA values are provided in the text.

Excluding allelic richness and expected heterozygosity, values of *A. stamineus* genetic diversity parameters obtained in 2009 were consistently positively correlated with measurements made at the same locations in 2011 at the site and watershed scales. Stronger relationships also were recovered for parameters based on nuclear microsatellite allele frequencies than mtDNA haplotype variation. Neither allelic richness nor expected heterozygosity were significantly correlated across years at the site or watershed scale (site: A_r, $r = 0.142$, $p = 0.340$; H_e, $r = 0.142$, $p = 0.340$; watershed: A_r, $r = 0.168$, $p = 0.358$; H_e, $r = 0.168$, $p = 0.358$). However, both Shannon diversity and the average number of alleles per locus were significantly correlated at the site (Shannon, $r = 0.437$, $p = 0.002$; NAA, $r = 0.439$, $p = 0.002$; Figure 45) and watershed scale (Shannon, $r = 0.506$, $p = 0.012$; NAA, $r = 0.546$, $p = 0.002$; Figure 45). An informative but non-significant relationship was recovered among mtDNA haplotype diversity at the site scale (site, r

= 0.211, p = 0.15) but not at the watershed scale (r = 0.028, p = 0.896; Figure 45). This indicates that measures of genetic diversity that are more strongly influenced by individual-level allelic variation (i.e., expected heterozygosity) vary over time, whereas measures that more strongly reflect population-level allelic variation (i.e., Shannon diversity) were generally stable across the study period. Measures based on haplotype variation appear to be more prone to sampling stochasticity, where the recovery of haplotypes may reflect the outcomes of random sampling from a large candidate pool (i.e., the number of haplotype recovered in a given year represents a fraction of the total number of haplotypes present in the overall population).

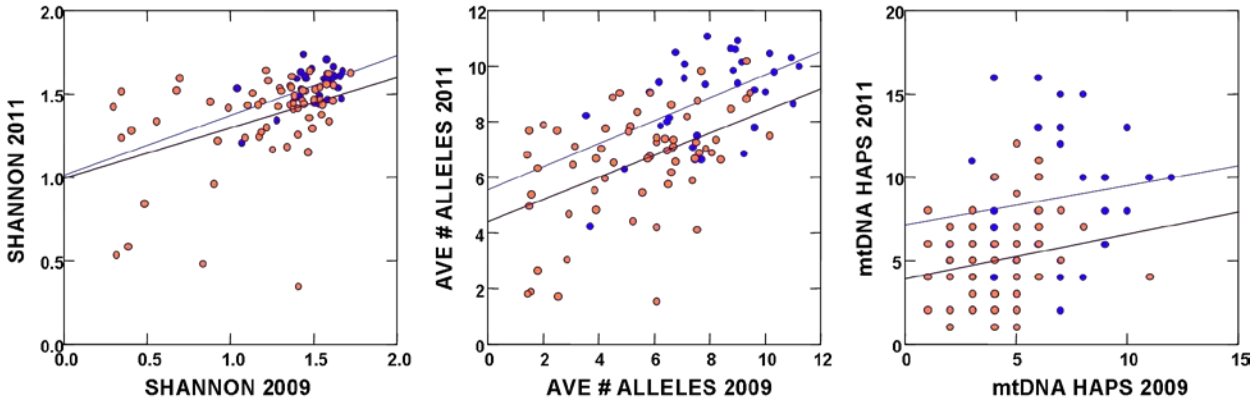

Figure 45: Comparison of *Awaous stamineus* nuclear microsatellite allelic diversity (Shannon diversity, average number of alleles per locus) and mitochondrial haplotype diversity (number of mtDNA haplotypes) in 2009 and 2011 for both site (orange and black) and watershed (blue) spatial scales. Correlation values are provided in the text.

Hereafter, results and discussion will focus solely on three measures of genetic diversity for *A. stamineus*: Shannon diversity, the average number of alleles per locus, and mtDNA haplotype diversity.

Genetic diversity in Sicyopterus: In 2009, *Sicyopterus stimpsoni* were sampled at 47 sites in 24 watersheds. Though sample sizes per watershed ranged as high as 45 individuals, ≤4 individuals were sampled at 23 sites, inclusive of both adults and postlarvae (Table 1). In 2011, *S. stimpsoni* were sampled at 42 sites in 23 watersheds, with sample sizes per watershed ranging from 1 to 79 individuals, inclusive of adults and postlarvae (Table 1). In 2011, only ten sites had samples sizes of ≤4 individuals. Nuclear genotype data were obtained for 239 individuals in 2009 and 768 individuals in 2011. A total of 431 alleles and an average of 53.8 alleles per locus were recovered from the genotyped individuals in 2009. A total of 574 alleles and an average of 68.1 alleles per locus were recovered from individuals sampled in 2011. Because of the frequently small number of total samples available for individual sites, subsequent analyses were restricted to watershed-scale comparisons.

The range of variation observed at measures of *S. stimpsoni* genetic diversity was generally greater for watersheds sampled in 2011 than 2009. Expected heterozygosity varied from 0.77 to 1 in 2009 and from 0.67 to 1 among all watersheds in 2011. Rarified allelic richness ranged from 1.77 to 2 and from 1.67 to 1.97 among all watersheds in 2009 and 2011, respectively. Shannon diversity of alleles varied from 0.55 to 3.01 and the average number of alleles per locus ranged from 1.8 to 24.6 among all watersheds in 2009. In 2011, Shannon diversity varied from 0.46 to

3.47 and the average number of alleles per locus ranged from 1.67 to 42.6. The lower maximum values of Shannon diversity and average number of alleles observe in 2009 are largely attributable to smaller sample sizes (overall, and per watershed).

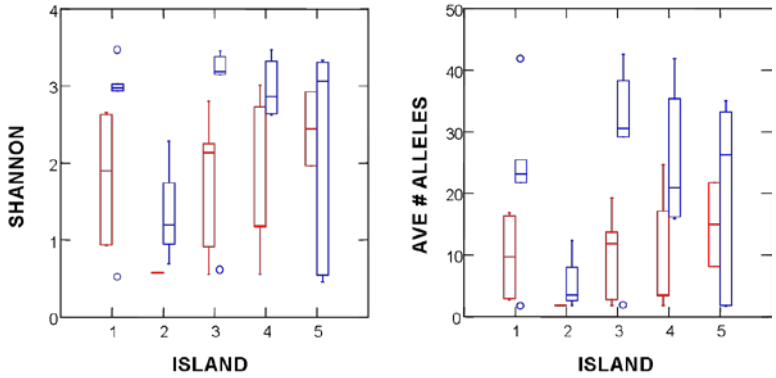

Figure 46: Comparison of watershed-scale estimates of *Sicyopterus stimpsoni* nuclear microsatellite allelic diversity (Shannon = Shannon diversity, Ave # alleles = average number of alleles per locus) in 2009 (red) and 2011 (blue) across islands (1 = Kauai, 2 = Oahu, 3 = Molokai, 4 = Maui, 5 = Hawaii). ANOVA values are provided in the text.

Though genetic diversity in *S. stimpsoni* varied across the archipelago (see section below on predictors of variation), no statistically significant differences were found among islands. This is likely attributable to the high levels of variation observed on each island (Figure 46). Post-hoc Tukey tests showed that differences among islands were not significant (ANOVA and pair-wise comparisons, all $p > 0.05$), but Shannon diversity and the average number of alleles per locus were consistently lower on Oahu than other islands in 2009 and 2011 (Figure 46). In 2009, maximum values of genetic diversity corresponded to watersheds on Maui, whereas maximum values corresponded to watersheds on Molokai in 2011 (Figure 46). Parallel findings were recovered for values averaged across years.

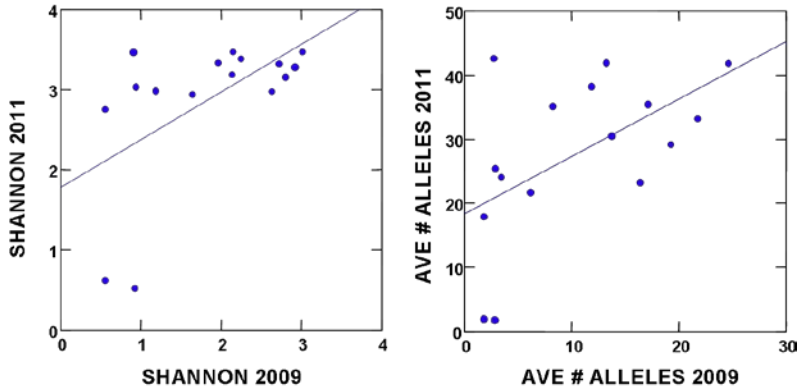

Figure 47: Comparison of *Sicyopterus stimpsoni* nuclear microsatellite allelic diversity (Shannon diversity, average number of alleles per locus) in 2009 and 2011 for watershed spatial scales. Correlation values are provided in the text.

Consistent relationships were recovered for *Sicyopterus stimpsoni* genetic diversity among watersheds sampled in 2009 and 2011. Informative, but statistically non-significant, relationships were recovered for allelic richness and expected heterozygosity (A_r, $r = 0.408$, $p = 0.116$; H_e, $r = 0.408$, $p = 0.116$). Both Shannon diversity of alleles and the average number of alleles per locus

were significantly positively correlated across years at the watershed scale (Shannon, r = 0.566, p = 0.022; NAA, r = 0.548, p = 0.028; Figure 47).

Population densities and diversity of native and non-native species: We observed all five native species on all five high islands. Though *L. concolor* was not observed in any survey conducted on Oahu in this study, it was observed on the island in a separate but related study (Hain et al. unpublished data). Across all sites sampled in 2009, we observed an average density per m^2 of 0.25 (range = 0-4.26) for *A. stamineus*, 0.05 (range = 0-0.53) for *E. sandwicensis*, 0.13 (range = 0-3.16) for *L. concolor*, 0.2 (range = 0-6.5) for *S. hawaiiensis*, 0.64 (range = 0-7.53) for *S. stimpsoni*, and 2.02 (range = 0-33.88) for Poeciliids. Waller-Duncan K-ratio *t*-tests ($\alpha = 0.05$) indicated informative but not significant differences in densities of *A. stamineus* between the islands of Kauai and Oahu (ANOVA; p = 0.0718, means = 0.4435 and 0.1035). On the other hand, *S. stimpsoni* were observed in significantly greater densities on Molokai (ANOVA; p < 0.0001), and greater densities of *S. hawaiiensis* were observed on Kauai (ANOVA; p = 0.0031). Observed densities of *L. concolor* were significantly greater on Kauai, Molokai and Maui (ANOVA; p = 0.0483, means = 0.2430, 0.3912, and 0.0860) than on Oahu and Hawaii (means = 0.0000 and 0.0326). Significant differences in observed densities were not detected among islands for *E. sandwicensis* or Poeciliids (ANOVA; p = 0.1288, p = 0.6216). Overall, goby densities were highest on Molokai, with densities on Oahu significantly lower than densities on Kauai or Molokai, and densities on Maui significantly lower than densities on Molokai (ANOVA; all p < 0.001). Native species richness (inclusive of all native fishes and amphidromous macroinvertebrates) was also significantly lower on Oahu in comparison to

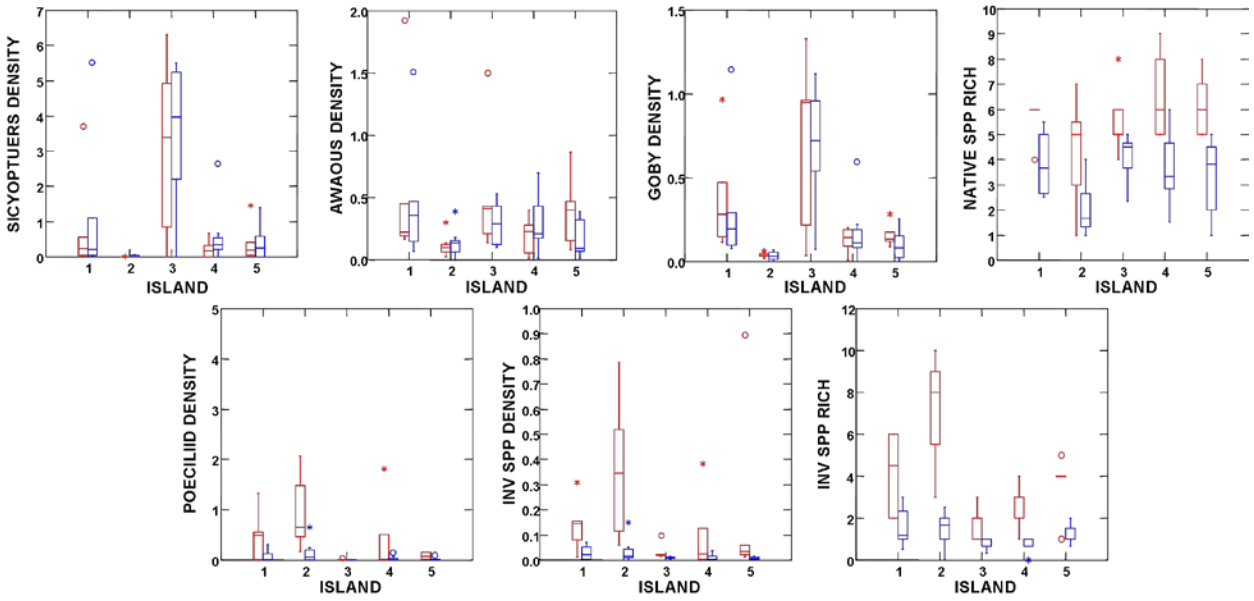

Figure 48: Comparison of watershed estimates of *Awaous stamineus*, Poeciliid, native goby and non-native species densities, native species richness and invasive species richness in 2009 (red) and 2011 (blue) across islands (1 = Kauai, 2 = Oahu, 3 = Molokai, 4 = Maui, 5 = Hawaii). ANOVA values are provided in the text.

Molokai and Hawaii (ANOVA; p = 0.008). Invasive species richness was significantly higher on Oahu and Kauai compared to Molokai and Maui (ANOVA; p < 0.001). Total species richness

varied among islands, with Maui supporting fewer species than other islands (ANOVA; p = 0.043).

Similar findings were observed for sites sampled in 2011 and for values averaged across years. In 2011, densities of *A. stamineus* differed among islands, particularly between Kauai and Oahu (ANOVA; p = 0.05). Densities of *S. stimpsoni* also differed among islands (ANOVA; p = 0.002), with the greatest change occurring between Molokai and Oahu (ANOVA; p = 0.001). Overall goby densities also differed among islands, with significantly greater densities occurring on Molokai (ANOVA; p < 0.001) and lower densities occurring on Oahu relative to Kauai. Native species richness was significantly lower on Oahu relative to other islands (ANOVA; p = 0.004). Significant differences in observed Poeciliids densities were not detected among islands (ANOVA; p = 0.10) or for cumulative invasive species densities (ANOVA; p = 0.17). Also, no differences in total species richness were observed among islands (ANOVA; p = 0.129), though Oahu exhibiting greater invasive species richness than Molokai and Maui (ANOVA; p = 0.026).

With few exceptions, site and watershed values of population- and assemblage-level parameters obtained in 2009 were significantly positively correlated with values obtained at the same locations in 2011(Figure 49). In general, stronger relationships were recovered for assemblage-level parameters in watershed-scale comparisons. Neither the density of *A. stamineus* nor total species richness were significantly correlated across years among watersheds ($density_{Awaous}$, r = 0.33, p = 0.09; SPR_{total}, r = -0.055, p = 0.340), though the density of *A. stamineus* was correlated among sites ($density_{Awaous}$, r = 0.341, p = 0.019; SPR_{total}, r = 0.228, p = 0.122). In contrast, the density of *S. stimpsoni* was significantly correlated across years among watersheds (r = 0.89, p < 0.001) and sites (r = 0.767, p < 0.001). The density of Poeciliids was not significantly correlated among sites or watersheds, though informative trends were recovered (site, r = 0.133, p = 0.375; watershed, r = 0.391, p = 0.06). The density of all gobies and all non-native

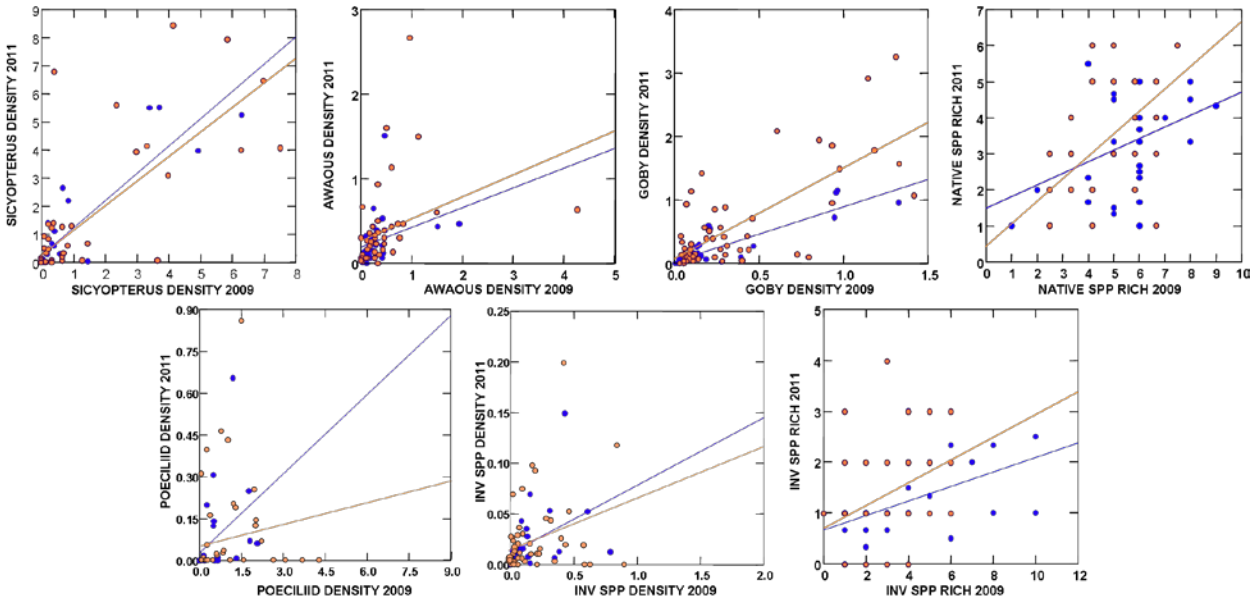

Figure 49: Comparison of *Sicyopterus stimpsoni*, *Awaous stamineus*, Poeciliid, native goby and non-native species densities, native species richness and invasive species richness in 2009 and 2011 for both watershed (blue) and site (orange) spatial scales. Correlation values are provided in the text.

species were significantly correlated across years among sites ($density_{goby}$, r = 0.676, p < 0.001; $density_{invasive}$, r = 0.310, p = 0.021) and watersheds ($density_{goby}$, r = 0.882, p < 0.001; $density_{invasive}$, r = 0.426, p = 0.027). Similarly, native species richness and invasive species richness were also significantly correlated across years among sites (SPR_{native}, r = 0.522, p < 0.001; $SPR_{invasive}$, r = 0.365, p = 0.012) and watersheds (SPR_{native}, r = 0.432, p = 0.035; $SPR_{invasive}$, r = 0.439, p = 0.032).

***Relationships between genetic diversity, goby population densities, and native species diversity*:** Significant positive correlations were consistently, though not always, recovered between measures of *Awaous stamineus* genetic diversity and *A. stamineus* population densities, whereas *A. stamineus* genetic diversity was not correlated with other measures of native population densities or species diversity. Positive or informative relationships were recovered between watershed-level estimates of Shannon diversity of alleles, the average number of alleles per locus, and mtDNA haplotype diversity in *A. stamineus* in comparison to *A. stamineus* population density in 2009 (Shannon, r = 0.348, p = 0.06; NAA, r = 0.472, p = 0.01; mtDNA, r = 0.392, p = 0.039; Figure 50). Estimates of *A. stamineus* genetic diversity were not significantly correlated with total goby density (Shannon, r = 0.150, p = 0.445; NAA, r = 0.141, p = 0.473; mtDNA, r = 0.30, p = 0.121; Figure 50) or native species richness (Shannon, r = 0.043, p = 0.827; NAA, r = 0.116, p = 0.555; mtDNA, r = 0.231, p = 0.238; Figure 50).

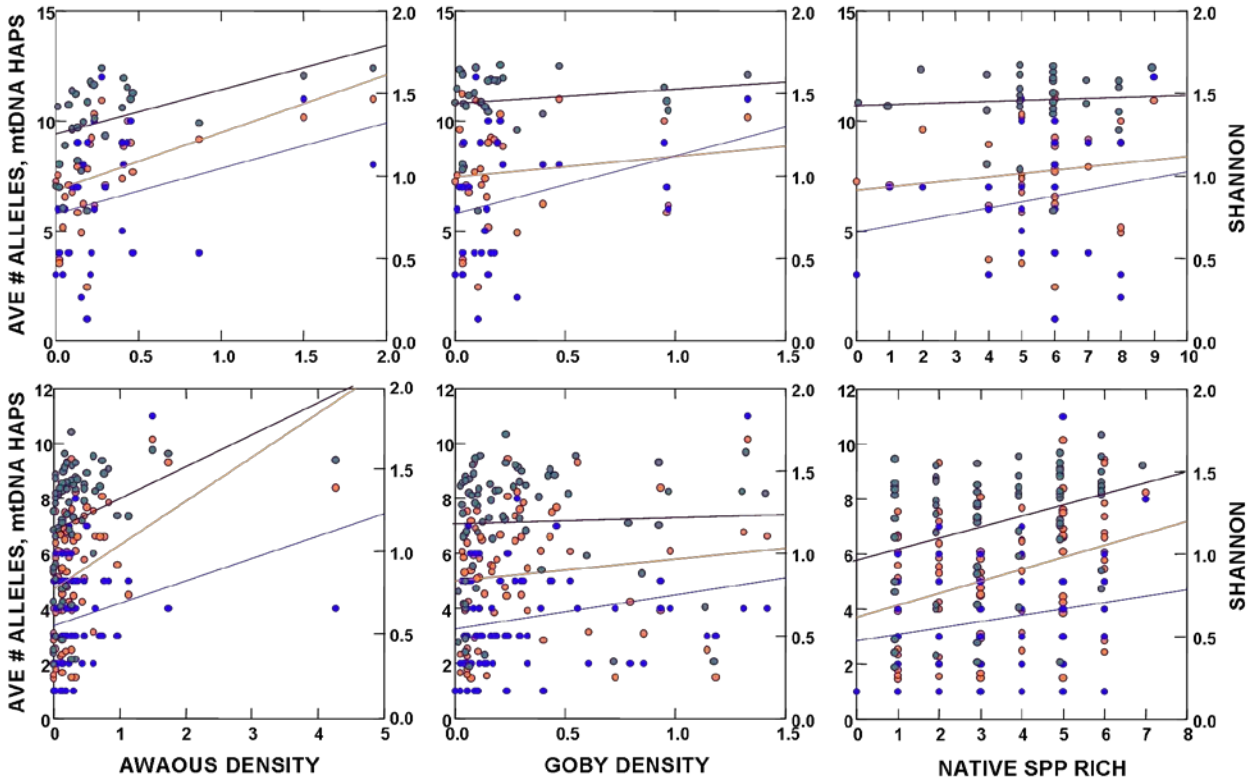

Figure 50: Comparison of *Awaous stamineus* genetic diversity to *A. stamineus* population density, native goby density, and native species richness in 2009 for both watershed (top) and site (bottom) spatial scales. Ave # alleles = average number of alleles per locus (orange), mtDNA Haps = number of mtDNA haplotypes (blue), Shannon = Shannon diversity of alleles (grey-green). Correlation values are provided in the text.

More informative relationships were recovered for site-level comparisons. Shannon diversity of alleles, the average number of alleles per locus, and mtDNA haplotype diversity in *A. stamineus* were positively related to *A. stamineus* population density (Shannon, r = 0.316, p = 0.008; NAA, r = 0.419, p < 0.001; mtDNA, r = 0.235, p = 0.05; Figure 50). Neither Shannon diversity nor the average number of alleles were correlated with total goby density (Shannon, r = 0.044, p = 0.719; NAA, r = 0.140, p = 0.253), but a correlation was recovered between mtDNA diversity and total goby density (r = 0.332, p = 0.005). Significant relationships also were recovered with native species richness (Shannon, r = 0.342, p = 0.002; NAA, r = 0.362, p = 0.001; mtDNA, r = 0.285, p = 0.015; Figure 50). Parallel, but slightly weaker relationships were found for values estimated from collections made in 2011. For example, estimates of genetic diversity in 2011 were not always significantly correlated with *A. stamineus* densities among sites (Shannon, r = 0.211, p = 0.082; NAA, r = 0.259, p = 0.032; mtDNA, r = 0.214, p = 0.077; Figure 50) or watersheds (Shannon, r = 0.286, p = 0.112; NAA, r = 0.319, p = 0.075; mtDNA, r = 0.257, p = 0.155; Figure 50).

In comparisons restricted to 2011 data, significant positive correlations were consistently but not always recovered between measures of *Sicyopterus stimpsoni* genetic diversity and *S. stimpsoni* population densities as well as other measures of native population densities and species diversity. Watershed-level estimates of Shannon diversity of alleles and the average number of alleles per locus were positively related to *S. stimpsoni* population density (Shannon, r = 0.501, p = 0.013; NAA, r = 0.674, p < 0.001; Figure 51). Estimates of genetic diversity also were significantly correlated with total goby density (Shannon, r = 0.501, p = 0.013; NAA, r = 0.663, p < 0.001; Figure 51) and with native species richness (Shannon, r = 0.520, p = 0.009; NAA, r = 0.497, p = 0.014; Figure 51). No significant correlations were recovered in comparisons restricted to 2009 data (all, p > 0.05), likely due to small sample sizes (overall, and per watershed). Thus, comparisons involving data from both years recovered positive or informative but non-significant relationships for estimates of Shannon diversity ($density_{Sicyopterus}$, r = 0.381, p = 0.042; $density_{Goby}$, r = 0.355, p = 0.05; SPR_{Native}, r = 0.331, p = 0.08) and the average number of alleles ($density_{Sicyopterus}$, r = 0.523, p = 0.004; $density_{Goby}$, r = 0.490, p = 0.007; SPR_{Native}, r = 0.308, p = 0.10).

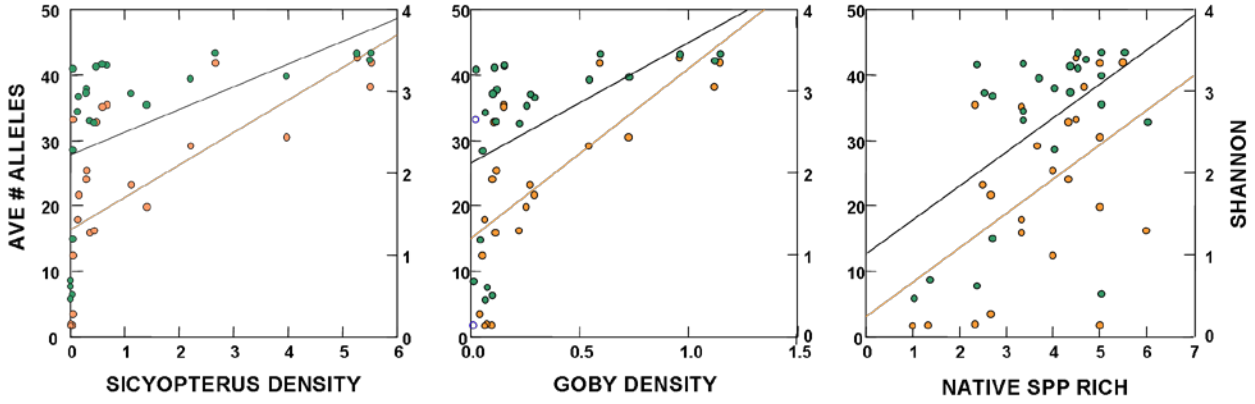

Figure 51: Comparison of *Sicyopterus stimpsoni* genetic diversity to *S. stimpsoni* population density, native goby density, and native species richness in 2011 at the watershed spatial scale. Ave # alleles = average number of alleles per locus (orange), Shannon = Shannon diversity of alleles (grey-green). Correlation values are provided in the text.

Estimates of population densities of native gobies were more often correlated with native species diversity across sites than across watersheds. In 2009 site-level and watershed-level comparisons, a significant relationship was recovered between *A. stamineus* population density and total goby density at both spatial scales (site, r = 0.362, p = 0.002; watershed, r = 0.527, p = 0.005; Figure 52). However, stronger relationships were generally recovered in site-level than watershed-level comparisons between *A. stamineus* population density and native species richness (site, r = 0.210, p = 0.084, watershed, r = 0.117, p = 0.552; Figure 52), and between total goby density and native species richness (site, r = 0.451, p < 0.001; watershed, r = 0.131, p = 0.508; Figure 52). In 2011 comparisons, a strong correlation was recovered between *A. stamineus* population density and total goby density across sites (r = 0.319, p = 0.008) and watersheds (r = 0.314, p = 0.08). However, no relationships between *A. stamineus* population density and native species richness were recovered (site, r = 0.097, p = 0.427; watershed, r = 0.107, p = 0.558), and a stronger correlation between total goby density and native species richness was recovered across watersheds (r = 0.385, p = 0.03) rather than sites (r = 0.226, p = 0.06). Positive relationships were recovered between *S. stimpsoni* population density and total goby density (Figure 53) at both spatial scales and both years (2009 site, r = 0.907, p < 0.001; 2009 watershed, r = 0.829, p < 0.001; 2011 site, r = 0.957, p < 0.001; 2011 watershed, r = 0.985, p < 0.001). With one exception, *S. stimpsoni* population density was also significantly correlated with native species richness at both spatial scales and both years (2009 site, r = 0.305, p = 0.009; 2009 watershed, r = 0.132, p = 0.457; 2011 site, r = 0.388, p < 0.001; 2011 watershed, r = 0.543, p < 0.001).

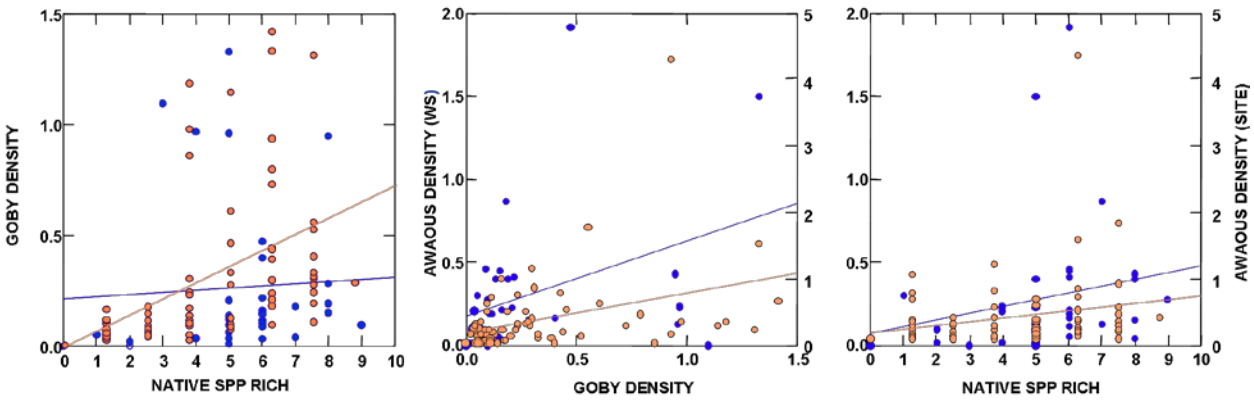

Figure 52: Comparison of native goby density to native species richness, and *Awaous stamineus* population density to native goby density and native species richness in 2009 for both site (orange) and watershed (blue) spatial scales. Correlation values are provided in the text.

***Predictors of Awaous stamineus genetic diversity*:** Pair-wise correlations indicate that in-stream and watershed conditions are strong predictors of *Awaous stamineus* genetic diversity, and cross-scale comparisons suggest that the strength of relationships likely reflects direct and indirect interactions. Comparisons restricted to data collected in 2009, for example, recovered negative correlations between watershed-scale measures of *A. stamineus* genetic diversity and the density

of invasive species (Shannon, r = -0.51, p = 0.003; NAA, r = -0.381, p = 0.034; mtDNA diversity, r = -0.263, p = 0.15). Stronger negative relationships were recovered with Poeciliid density (Shannon, r = -0.606, p = 0.001; NAA, r = -0.443, p = 0.018; mtDNA diversity, r = -0.293, p = 0.13), which are among the most prevalent invasive species in Hawaii. In contrast, site-level comparisons did not recover significant relationships (all, p > 0.05). This is consistent with other data suggesting that the presence and density of Poeciliids in a watershed, rather than at individual sites, is a stronger determinant of effects on native species (see section 5.1.3).

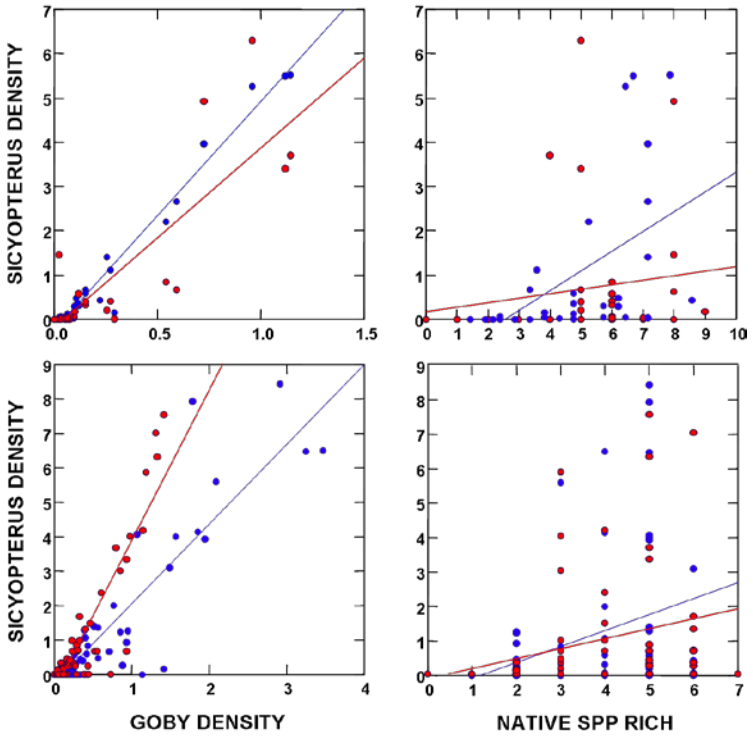

Figure 53: Comparison of *Sicyopterus stimpsoni* population density to native goby density and native species richness in 2009 (red) and 2011 (blue) at the watershed (top) and site (bottom) spatial scales. Correlation values are provided in the text.

Informative, but non-significant relationships were recovered between most measures of *A. stamineus* genetic diversity and watershed-scale land use intensification (Shannon, r = -0.230, p = 0.22; NAA, r = -0.219, p = 0.245; mtDNA diversity, r = 0.057, p = 0.765). Either negative or no relationship was recovered with impervious surface in lower watershed reaches (Shannon, r = -0.35, p = 0.05; NAA, r = -0.257, p = 0.171; mtDNA diversity, r = 0.008, p = 0.996). Site-level comparisons recovered both significant negative and informative relationships with watershed land use intensification (Shannon, r = -0.214, p = 0.08; NAA, r = -0.203, p = 0.09; mtDNA diversity, r = -0.074, p = 0.535) and impervious surfaces in lower watershed reaches (Shannon, r = -0.289, p = 0.017; NAA, r = -0.248, p = 0.042; mtDNA diversity, r = -0.137, p = 0.249). This

provides further evidence suggesting that lower reaches may represent biological gauntlets (i.e., impaired areas that all immigrants and emigrants must pass through). No significant or informative relationships were recovered with water chemistry or stable isotope parameters either at the site or watershed scale (all, $p > 0.05$).

Cross-year comparisons suggest that the strength of pair-wise relationships also can vary across time. Watershed- and site-level comparisons restricted to data collected in 2011 did not recover significant relationships between measures of *A. stamineus* genetic diversity and invasive species density or Poeciliid densities (all, $p > 0.05$). This is not particularly surprising since we also found that Poeciliid densities were not correlated between years (though invasive species densities were correlated; see text above). However, relationships recovered between measures of *A. stamineus* genetic diversity and land use conditions were generally consistent with those detected for 2009. Watershed-level comparisons recovered non-significant relationships with watershed-level land use intensification (Shannon, $r = -0.223$, $p = 0.273$; NAA, $r = -0.239$, $p = 0.24$; mtDNA diversity, $r = -0.088$, $p = 0.667$), though negative relationships were recovered with impervious surfaces in lower watershed reaches (Shannon, $r = -0.411$, $p = 0.018$; NAA, $r = -0.448$, $p = 0.009$; mtDNA diversity, $r = -0.234$, $p = 0.189$). Similar relationships were recovered in site-level comparisons (impervious surface in the lower watershed; Shannon, $r = -0.452$, $p < 0.001$; NAA, $r = -0.268$, $p = 0.026$). Again, no strong relationships were observed with water chemistry or stable isotope parameters (all, $p > 0.05$).

Pair-wise comparisons based on 2009 and 2011 data provide some perspective of relationships integrated over time and space. Negative relationships were recovered between most watershed-scale measures of genetic diversity and the density of invasive species (Shannon, $r = -0.359$, $p = 0.034$; NAA, $r = -0.314$, $p = 0.06$; mtDNA diversity, $r = -0.16$, $p = 0.358$). Informative, but non-significant relationships were recovered for Poeciliid densities (Shannon, $r = -0.241$, $p = 0.163$; NAA, $r = -0.22$, $p = 0.204$; mtDNA diversity, $r = -0.179$, $p = 0.303$). No relationships were detected in site-level comparisons (all, $p > 0.05$). Negative relationships also were consistently recovered with impervious surfaces in lower reaches (watershed, Shannon, $r = -0.41$, $p = 0.015$; NAA, $r = -0.338$, $p = 0.047$; mtDNA, $r = -0.063$, $p = 0.72$; and sites, Shannon, $r = -0.284$, $p = 0.003$; NAA, $r = -0.237$, $p = 0.014$; mtDNA, $r = -0.038$, $p = 0.698$) and for site-level comparisons of broader measures of land use intensification (e.g., PC1 watershed, Shannon, $r = -0.196$, $p = 0.259$; NAA, $r = -0.179$, $p = 0.303$; mtDNA, $r = -0.038$, $p = 0.83$; and sites, Shannon, $r = -0.221$, $p = 0.023$; NAA, $r = -0.187$, $p = 0.05$; mtDNA, $r = -0.029$, $p = 0.771$). Consistent with the analyses of 2009 and 2011 data, no relationships were observed with water chemistry or stable isotope parameters (all, $p > 0.05$).

Stepwise regression models, which accounted for covariance among in-stream and watershed conditions, consistently identified *A. stamineus* population density as being a predictor of *A. stamineus* genetic diversity among watersheds (Tables 9 and 10). Watershed area, distance to the stream mouth, land use intensification, canopy cover, and phosphorus-driven water chemistry gradients also were identified as predictors, with most of the additional factors identified in models of 2011 data. When *A. stamineus* density was excluded from consideration, stepwise regression identified watershed area and Poeciliid density as significant predictors in 2009. Watershed area, distance to the stream mouth, canopy cover, and nutrient loading were identified

as predictors in models of 2011 data. No other variables were identified in models of data from both years.

Stepwise regression models identified a wider range of predictors of *A. stamineus* genetic diversity among sites (Tables 9 and 10). In addition to *A. stamineus* density, both watershed land

2009

	Shannon		NAA		mtDNA		Awaous dens	Poec dens	Native SPP		Inv SPP		Total SPP	
Watershed area					0.380 (+) 0.058		0.518 (+) 0.003							
Distance to Mouth							0.247 (-) 0.138	0.293 (+) 0.061	0.301 (-) 0.047	0.301 (-) 0.047	0.517 (-) 0.011	0.517 (-) 0.011		
LULC PC1								0.491 (+) 0.003	0.304 (-) 0.047	0.304 (-) 0.047				
LULC PC2								0.332 (-) 0.032			0.44 (-) 0.075	0.44 (-) 0.075		
Water Chemistry PC1									0.253 (-) 0.129	0.253 (-) 0.129	0.518 (+) 0.001	0.518 (+) 0.001		
Water Chemistry PC2														
Poeciliid Density		0.606 (-) 0.001									1.054 (+) 0.001	1.054 (+) 0.001		
A. stamineus Density	0.363 (+) 0.045		0.481 (+) 0.006		0.492 (+) 0.016									
Full Model	0.363 0.045	0.606 0.001	0.481 0.006		0.453 0.04		0.504 0.011	0.673 0.001	0.65 0.001	0.65 0.001	0.808 <0.001	0.808 <0.001		

2011

	Shannon		NAA		mtDNA		Awaous dens	Poec dens	Native SPP		Inv SPP		Total SPP	
Watershed area		0.441 (+) 0.02		0.451(+) 0.02										
Distance to Mouth	0.368 (-) 0.028		0.336 (-) 0.032			0.374 (-) 0.036								
LULC PC1					0.27(-) 0.112			0.401 (+) 0.021	0.779 (-) <0.001	0.729 (-) <0.001			0.365 (-) 0.037	0.365 (-) 0.037
LULC PC2		0.422 (+) 0.03		0.44 (+) 0.02	0.334 (+) 0.064	0.253 (+) 0.14								
Water Chemistry PC1		0.422 (-) 0.03		0.356 (-) 0.072							0.372 (+) 0.073	0.372 (+) 0.073		
Water Chemistry PC2					0.334 (+) 0.054		0.356 (+) 0.088							
Poeciliid Density														
A. stamineus Density	0.328 (+) 0.049		0.456 (+) 0.005		0.408 (+) 0.032				0.240 (+) 0.109					
Full Model	0.536 0.007	0.617 0.02	0.615 0.001	0.626 0.017	0.724 0.005	0.449 0.084	0.356 0.088	0.401 0.021	0.766 <0.001	0.729 <0.001	0.372 0.073	0.372 0.073	0.365 0.037	0.365 0.037

Combined

	Shannon		NAA		mtDNA		Awaous dens	Poec dens	Native SPP		Inv SPP		Total SPP	
Watershed area							0.464 (+) 0.005				0.179 (+) 0.15	0.179 (+) 0.15		
Distance to Mouth							0.300 (-) 0.06	0.616 (+) <0.001						
LULC PC1								0.399 (+) <0.001	0.332 (-) 0.054	0.332 (-) 0.054				
LULC PC2					0.229 (+) 0.15			0.275 (-) <0.001						
Water Chemistry PC1								0.196 (+) 0.036			0.414 (+) 0.004	0.414 (+) 0.004		
Water Chemistry PC2														
Poeciliid Density									0.363 (-) 0.036	0.363 (-) 0.036	0.386 (+) 0.007	0.386 (+) 0.007		
A. stamineus Density	0.305 (+) 0.071		0.418 (+) 0.01		0.355 (+) 0.035									
Full Model	0.305 0.071		0.418 0.01		0.394 0.062		0.462 0.012	0.892 <0.001	0.387 0.045	0.387 0.045	0.703 <0.001	0.703 <0.001		

Table 9: Results of stepwise regression of 2009, 2011, and combined watershed-level estimates of *Awaous stamineus* Shannon diversity (Shannon), average number of alleles per locus (Naa), mtDNA haplotype diversity (mtDNA), *A. stamineus* population density (Awaous dens), Poeciliid density (Poec dens), native species richness (Native SPP), non-native species richness (Inv SPP), and total species richness (Total SPP) against physiographic, abiotic environmental, and biotic factors. LULC = land use. Lefthand column models are inclusive of *A. stamineus* density as an explanatory factor; righthand column models exclude *A. stamineus* density. Regression models of *A. stamineus* density exclude *A. stamineus* density as an explanatory factor, and similarly regression models of Poeciliid density exclude Poeciliid density as an explanatory factor. Top values reported in cells are partial correlation coefficients, with the direction of the relationship given as either positive (+) or negative (-). Full model r^2 values are also given in the second to bottom row of each table. Bottom values reported in cells are p-values.

use (intensification and canopy cover) and water chemistry conditions (nitrogen and phosphorus gradients) were recovered as predictors in models of 2009 data. In contrast, fewer variables were identified as predictors in site-level models of 2011 data. Only distance to the stream mouth and

2009

	Shannon		NAA		mtDNA		Awaous dens	Poec dens	Native SPP		Inv SPP		Total SPP	
Watershed area		0.213 0.072		0.309 (+) 0.008			0.542 (+) <0.001		0.224 (+) 0.017	0.224 (+) 0.017			0.297 (+) 0.011	0.297 (+) 0.011
Distance to Mouth									0.370 (-) <0.001	0.370 (-) <0.001	0.182 (+) 0.11	0.182 (+) 0.11	0.226 (-) 0.05	0.226 (-) 0.05
LULC PC1	0.181 (-) 0.109	0.210 (-) 0.072		0.196 (-) 0.085				0.444 (+) <0.001	0.275 (-) 0.011	0.275 (-) 0.011	0.267 (+) 0.028	0.267 (+) 0.028		
LULC PC2	0.212 0.064	0.200 (+) 0.092		0.216 0.062										
Water Chemistry PC1			0.159 (-) 0.141								0.22 (+) 0.056	0.22 (+) 0.056		
Water Chemistry PC2			0.228 (+) 0.038											
Poeciliid Density									0.213 (-) 0.039	0.213 (-) 0.039				
A. stamineus Density	0.323 (+) 0.006		0.429 (+) <0.001		0.250 (+) 0.033									
Full Model	0.417 0.005	0.343 0.038	0.496 <0.001	0.402 0.008	0.250 (+) 0.033		0.542 (+) <0.001	0.444 <0.001	0.667 <0.001	0.667 <0.001	0.478 <0.001	0.478 <0.001	0.345 0.12	0.345 0.12

2011

	Shannon		NAA		mtDNA		Awaous dens	Poec dens	Native SPP		Inv SPP		Total SPP	
Watershed area														
Distance to Mouth					0.212 (-) 0.068				0.18 (-) 0.128	0.18 (-) 0.128				
LULC PC1	0.328 (-) 0.004						0.239 (-) 0.088	0.255 (+) 0.025	0.579 (-) <0.001	0.579 (-) <0.001			0.597(-) <0.001	0.597(-) <0.001
LULC PC2		0.263 (+) 0.062	0.269 (+) 0.065	0.277 (+) 0.049		0.235 (+) 0.098			0.229 (+) 0.052	0.229 (+) 0.052			0.256 (+) 0.034	0.256 (+) 0.034
Water Chemistry PC1									0.171 (+) 0.15	0.171 (+) 0.15			0.238 (+) 0.051	0.238 (+) 0.051
Water Chemistry PC2							0.463 (-) 0.001							
Poeciliid Density							0.199 (-) 0.15				0.408 (+) <0.001	0.408 (+) <0.001	0.242 (+) 0.047	0.242 (+) 0.047
A. stamineus Density														
Full Model	0.328 0.004	0.263 0.062	0.269 0.065	0.277 0.049	0.212 0.068	0.235 0.098	0.487 0.007	0.255 0.025	0.642 <0.001	0.642 <0.001	0.408 <0.001	0.408 <0.001	0.631 <0.001	0.631 <0.001

Combined

	Shannon		NAA		mtDNA		Awaous dens	Poec dens	Native SPP		Inv SPP		Total SPP	
Watershed area		0.135 0.15		0.193 (+) 0.041		0.219 (+) 0.023	0.533 (+) <0.001			0.245 (+) 0.007			0.248 (+) 0.008	0.248 (+) 0.008
Distance to Mouth								0.136 (+) 0.15		0.148 (-) 0.113	0.244 (+) 0.003	0.244 (+) 0.003		
LULC PC1	0.205 (-) 0.026	0.213 (-) 0.025	0.171 (-) 0.057	0.181 (-) 0.055				0.266 (+) 0.006	0.365 (-) <0.001	0.331 (-) <0.001			0.250 (-) 0.016	0.250 (-) 0.016
LULC PC2														
Water Chemistry PC1											0.238 (+) 0.004	0.238 (+) 0.004	0.157 (+) 0.114	0.157 (+) 0.114
Water Chemistry PC2	0.144 (-) 0.117	0.170 (-) 0.072	0.124 (-) 0.15	0.154 (-) 0.101										
Poeciliid Density											0.407 (+) <0.001	0.407 (+) <0.001	0.196 (+) 0.042	0.196 (+) 0.042
A. stamineus Density	0.273 (+) 0.003		0.340 (+) <0.001		0.397 (+) <0.001				0.216 (+) 0.015					
Full Model	0.387 0.001	0.308 0.015	0.418 <0.001	0.313 0.013	0.397 <0.001	0.219 0.023	0.533 <0.001	0.327 0.003	0.437 <0.001	0.456 <0.001	0.575 <0.001	0.575 <0.001	0.386 0.002	0.386 0.002

Table 10: Results of stepwise regression of 2009, 2011, and combined site-level estimates of *Awaous stamineus* Shannon diversity (Shannon), average number of alleles per locus (Naa), mtDNA haplotype diversity (mtDNA), *A. stamineus* population density (Awaous dens), Poeciliid density (Poec dens), native species richness (Native SPP), non-native species richness (Inv SPP), and total species richness (Total SPP) against physiographic, abiotic environmental, and biotic factors. LULC = land use. Lefthand column models are inclusive of *A. stamineus* density as an explanatory factor; righthand column models exclude *A. stamineus* density. Regression models of *A. stamineus* density exclude *A. stamineus* density as an explanatory factor, and similarly regression models of Poeciliid density exclude Poeciliid density as an explanatory factor. Top values reported in cells are partial correlation coefficients, with the direction of the relationship given as either positive (+) or negative (-). Full model r^2 values are also given in the second to bottom row of each table. Bottom values reported in cells are p-values.

watershed land use (intensification and canopy cover) were identified, regardless of whether *A. stamineus* density was included in the model. A consistent set of predictors- including *A. stamineus* density, land use intensification, and phosphorus gradients- were identified in models of data from both years. With *A. stamineus* density excluded, cross-year models identified the same set of predictors, with watershed area replacing *A. stamineus* density.

2009

	Shannon		NAA		Sicy dens
Watershed area					
Distance to Mouth	0.554 (+) 0.045	0.538 (+) 0.095			
LULC PC1	0.621 (-) 0.027				
LULC PC2				0.650 (+) 0.011	
Water Chemistry PC1				0.470 (+) 0.005	
Water Chemistry PC2					
Poeciliid Density		0.689 (-) 0.038		0.813 (-) 0.001	0.438 (-) 0.029
S. stimpsoni Density			0.674 (+) <0.001		
Full Model	0.532 0.059	0.511 0.103	0.674 <0.001	0.769 0.005	0.438 0.029

2011

	Shannon		NAA		Sicy dens
Watershed area	0.265 (+) 0.14				
Distance to Mouth	0.347 (+) 0.069		0.382 (+) 0.034		
LULC PC1				0.339 (-) 0.149	0.319 (-) 0.071
LULC PC2	0.25 (+) 0.136				
Water Chemistry PC1					
Water Chemistry PC2					
Poeciliid Density	0.308 (-) 0.094	0.395 (-) 0.056		0.359 (-) 0.128	
S. stimpsoni Density	0.541 (+) 0.01		0.812 (+) <0.001		
Full Model	0.847 <0.001	0.395 0.056	0.793 <0.001	0.557 0.009	0.319 0.071

Combined

	Shannon		NAA		Sicy dens
Watershed area					
Distance to Mouth		0.486 (+) 0.038	0.358 (+) 0.086	0.336 (+) 0.14	
LULC PC1					0.316 (-) 0.038
LULC PC2					
Water Chemistry PC1					
Water Chemistry PC2					0.365 (+) 0.018
Poeciliid Density		0.636 (-) 0.008	0.511 (-) 0.021	0.646 (-) 0.007	
S. stimpsoni Density	0.381 (+) 0.042		0.426 (+) 0.017		
Full Model	0.381 0.042	0.504 0.026	0.6464 0.004	0.508 0.024	0.477 0.01

Predictors of Sicyopterus stimpsoni genetic diversity: Pair-wise negative correlations were consistently recovered between watershed-scale estimates of *Sicyopterus stimpsoni* genetic diversity and conditions associated with biotic and abiotic stream degradation. Comparisons restricted to data collected in 2009 recovered informative but non-significant relationships with Poeciliid density (Shannon, r = -0.204, p = 0.389; NAA, r = -0.306, p = 0.189) and invasive species density (Shannon, r = -0.187, p = 0.431; NAA, r = -0.314, p = 0.177). Similar relationships were recovered in comparisons restricted to data from 2011 (Poeciliid density, Shannon, r = -0.372, p = 0.117; NAA, r = -0.334, p = 0.163; Invasive species density, Shannon, r = -0.242, p = 0.318; NAA, r = -0.247, p = 0.308). Informative but non-significant relationships were recovered between measures of *S. stimpsoni* genetic diversity and watershed-scale land use intensification (2009, Shannon, r = -0.291, p = 0.213; NAA, r = -0.388, p = 0.09; 2011, Shannon, r = -0.192, p = 0.432; NAA, r = -0.330, p = 0.167). Unlike the trends detected for *A. stamineus*, negative relationships were not consistently recovered with land use intensification at the mouth of watersheds (2009, Shannon, r = -0.021, p = 0.930; NAA, r = -0.056, p = 0.813; 2011, Shannon, r = -0.380, p =

Table 11: Results of stepwise regression of 2009, 2011, and combined watershed-level estimates of *Sicyopterus stimpsoni* Shannon diversity (Shannon), average number of alleles per locus (NAA), mtDNA haplotype diversity (mtDNA), *S. stimpsoni* population density (Sicy dens), against physiographic, abiotic environmental, and biotic factors. LULC = land use. Lefthand column models are inclusive of *S. stimpsoni* density as an explanatory factor; righthand column models exclude *S. stimpsoni* density. Regression models of *S. stimpsoni* density exclude *S. stimpsoni* density as an explanatory factor. Top values reported in cells are partial correlation coefficients, with the direction of the relationship given as either positive (+) or negative (-). Full model r^2 values are also given in the second to bottom row. Bottom values reported in cells are p-values.

0.109; NAA, r = -0.457, p = 0.049), which suggests that *S. stimpsoni* may experience weaker indirect effects arising from impairment of lower reaches in a watershed (i.e., lower reaches may not represent biological gauntlets that all immigrants and emigrants must pass through), or that the strength of indirect effects from lower watershed impairment varies over time.

In contrast to relationships observed for *A. stamineus*, significant pair-wise correlations were recovered between *S. stimpsoni* genetic diversity, water chemistry and stable isotope parameters. No informative relationships were detected when comparisons were restricted to data from 2009 (all, p > 0.05), but when comparisons were restricted to data from 2011, consistent negative relationships were recovered between genetic diversity and indicators of nutrient loading (PC1; Shannon, r = -0.248, p = 0.392; NAA, r = -0.338, p = 0.245; goby δ^{15}N, Shannon, r = -0.406, p = 0.007; NAA, r = -0.448, p = 0.002) and parameters associated with sedimentation like total suspended solids (Shannon, r = -0.410, p = 0.145; NAA, r = -0.478, p = 0.084).

	Sicy dens 2009	Sicy dens 2011
Watershed area		
Distance to Mouth	0.19 (-) 0.114	
LULC PC1	0.225 (-) 0.062	0.327 (-) 0.017
LULC PC2		
Water Chemistry PC1		
Water Chemistry PC2		0.295 (+) 0.03
Poeciliid Density		
S. stimpsoni Density		
Full Model	0.337 0.015	0.419 0.01

Table 12: Results of stepwise regression of 2009 and 2011 site-level estimates of *Sicyopterus stimpsoni* population density (Sicy dens) against physiographic, abiotic environmental, and biotic factors. LULC = land use. Regression models exclude *S. stimpsoni* density as an explanatory factor. Top values reported in cells are partial correlation coefficients, with the direction of the relationship given as either positive (+) or negative (-). Full model r^2 values are also given in the second to bottom row. Bottom values reported in cells are p-values.

With few exceptions, comparisons involving data from both 2009 and 2011 detected parallel relationships with parameters associated with biotic and abiotic degradation of stream conditions. Relationships were generally weaker, likely because of counterbalancing trends arising from the 2009 and 2011 data, respectively. Negative and informative relationships were recovered with Poeciliid density (Shannon, r = -0.323, p = 0.108; NAA, r = -0.444, p = 0.023) and invasive species density (Shannon, r = -0.352, p = 0.078; NAA, r = -0.434, p = 0.027). Similarly, informative relationships were recovered with watershed-scale (Shannon, r = -0.251, p = 0.217; NAA, r = -0.357, p = 0.073) and lower watershed land use intensification (Shannon, r = -0.243, p = 0.232; NAA, r = -0.369, p = 0.064), as well as parameters associated with nutrient loading (PC1, Shannon, r = -0.151, p = 0.461; NAA, r = -0.260, p = 0.20). No clear trends were detected with conditions associated with sedimentation (e.g., TSS, Shannon, r = -0.047, p = 0.819; NAA, r = -0.116, p = 0.574).

Stepwise regression models consistently identified *S. stimpsoni* population density, Poeciliid density, and distance to the stream mouth as predictors of *S. stimpsoni* genetic diversity among watersheds (Tables 11 and 12). Models restricted to data from 2009 also identified land use intensification as a predictor of *S. stimpsoni* genetic diversity, and models of 2011 data identified watershed area and canopy cover as predictor variables. When *S. stimpsoni* density was excluded from consideration, canopy cover and nitrogen-driven gradients in water quality were identified as predictors of *S. stimpsoni* genetic diversity in 2009. When *S. stimpsoni* density was excluded from 2011 models, a broader array of parameters was identified, including: watershed area, distance to the stream mouth, canopy cover, and nitrogen-driven gradients in water quality. Models of data from both years

only identified *S. stimpsoni* density, Poeciliid density, and distance to the stream mouth as predictors of *S. stimpsoni* genetic diversity (Tables 11 and 12).

Predictors of population densities and species richness: Pair-wise comparisons indicate that in-stream and watershed conditions are strong predictors of native and non-native population densities. No significant or informative pair-wise correlations were recovered for *Awaous stamineus* densities in annual or combined analyses (all, $p > 0.05$), which is consistent with the species having a broad geographic distribution and moderate tolerance of impaired conditions. However, negative pair-wise correlations were consistently recovered for *S. stimpsoni* population densities and conditions associated with degradation, with stronger relationships more often recovered for 2011 than 2009. In both watershed and site-level comparisons, negative but largely non-significant correlations were detected with Poeciliid density (2009 watershed, $r = -0.366$, $p = 0.113$; 2009 site, $r = -0.247$, $p = 0.037$; 2011 watershed, $r = -0.249$, $p = 0.305$, 2011 site, $r = -0.222$, $p = 0.10$). Similarly, negative and informative correlations were recovered between *S. stimpsoni* population densities and land use intensification (2009 watershed, $r = -0.350$, $p = 0.13$; 2009 site, $r = -0. 285$, $p = 0.015$; 2011 watershed, $r = -0.360$, $p = 0.130$, 2011 site, $r = -0.281$, $p = 0.036$). Stronger negative relationships were recovered with intensification in the lower watershed (2009 watershed, $r = -0.448$, $p = 0.05$; 2009 site, $r = - 0.245$, $p = 0.038$; 2011 watershed, $r = -0.353$, $p = 0.09$, 2011 site, $r = -0.239$, $p = 0.036$), as well as stable isotope measures of nutrient loading (e.g., goby $\delta^{15}N$ 2009 watershed, $r = -0.600$, $p = 0.014$; 2009 site, $r = - 0.439$, $p = 0.017$; 2011 watershed, $r = -0.455$, $p = 0.10$, 2011 site, $r = -0.475$, $p = 0.019$), and sedimentation (e.g., TSS, 2009 watershed, $r = -0.436$, $p = 0.091$; 2009 site, $r = -0.278$, $p = 0.145$; 2011 watershed, $r = -0.406$, $p = 0.149$, 2011 site, $r = -0.305$, $p = 0.148$).

Relationships recovered for total goby densities largely paralleled those found for *S. stimpsoni* densities. Negative relationships were recovered with Poeciliid density (2009 watershed Poeciliids, $r = -0.357$, $p = 0.06$; 2009 site Poeciliids, $r = - 0.267$, $p = 0.026$; 2011 watershed Poeciliids, $r = -0.173$, $p = 0.345$, 2011 site Poeciliids, $r = -0.243$, $p = 0.104$). Negative relationships also were recovered with land use intensification (e.g., 2009 watershed, impervious surfaces in lower reaches, $r = -0.279$, $p = 0.11$; 2009 site, $r = - 0.281$, $p = 0.02$; 2011 watershed, impervious, $r = -0.192$, $p = 0.293$, 2011 site, $r = -0.262$, $p = 0.079$), as well as measures of nutrient loading (e.g., 2009 watershed goby $\delta^{15}N$, $r = -0.483$, $p = 0.027$; 2009 site goby $\delta^{15}N$, $r = -0.319$, $p = 0.091$; 2011 watershed goby $\delta^{15}N$, $r = -0.424$, $p = 0.071$, 2011 site goby $\delta^{15}N$, $r = -0.321$, $p = 0.029$).

Pair-wise relationships recovered between native species richness and invasive species, land use, and water chemistry conditions were similar to those recovered for *S. stimpsoni* densities and total goby densities. Stronger negative relationships with Poeciliid density were recovered in 2009 than 2011 (2009 watershed Poeciliids, $r = -0.36$, $p = 0.05$; 2009 site Poeciliids, $r = -0.418$, $p < 0.001$; 2011 watershed Poeciliids, $r = -0.094$, $p = 0.608$, 2011 site Poeciliids, $r = 0.1$, $p = 0.734$). Negative relationships were also recovered for isotopic measures of nutrient loading (e.g., 2009 watershed goby $\delta^{15}N$, $r = -0.42$, $p = 0.061$; 2009 site goby $\delta^{15}N$, $r = -0.219$, $p = 0.255$; 2011 watershed goby $\delta^{15}N$, $r = -0.548$, $p = 0.015$, 2011 site goby $\delta^{15}N$, $r = -0.559$, $p = 0.007$).

Physiographic and land use conditions were consistently identified as predictors of population densities and species richness in watershed-scale stepwise regression models (Table 9).

Watershed area and distance to the stream mouth were identified as predictors of *A. stamineus* density in models of the 2009 and combined data sets. Distance to the stream mouth also was a significant predictor of Poeciliid density, native species richness, and invasive species richness in models of the 2009 data. Land use intensification was a predictor of *S. stimpsoni* density in the model of the 2011 data as well as Poeciliid density and native species richness in models of the 2009, 2011 and combined data sets. It was also the only predictor of total species richness, having been retained in the model of 2011 data. Canopy cover was identified as a predictor of Poeciliid density in models of the 2009 and combined data sets, as well as invasive species richness in models of the 2009 data. Water chemistry and population density parameters were less often identified as predictors (Table 9), though nitrogen-driven gradients in water quality were consistently retained as a predictor of invasive species richness, native species richness and Poeciliid density. Phosphorus-driven gradients in water chemistry was the only predictor identified for the 2011 data on *A. stamineus* density. Poeciliid density was a significant predictor of *S. stimpsoni* density in the model of the 2009 data as well as invasive species richness in models of 2009 and combined data. *A. stamineus* density was retained as a predictor of native species richness in the model of 2011 data.

Similar sets of predictors were identified in site-level stepwise regression models of native and non-native population densities and species richness (Table 10). As in watershed-scale models, physiographic and land use conditions were consistently identified as predictors. Watershed area was a contributing driver of *A. stamineus* density, native species richness, and total species richness in models of the 2009 and combined data sets. Distance to the stream mouth was consistently identified as a predictor of *S. stimpsoni* density, Poeciliid density, native species richness, and invasive species richness. Similarly, land use intensification was a contributing driver of *A. stamineus* density, *S. stimpsoni* density, Poeciliid density, native species richness, and total species richness. It was also identified as a predictor of invasive species richness in models of the 2011 data. Canopy cover was identified as a predictor of native and total species richness in models of the 2011 data. As with watershed-scale models, water chemistry and population densities were less often identified as predictors in site-level models (Table 10). Nitrogen-driven gradients in water quality were identified as a predictor of invasive species richness in models of the 2009 and combined data, as well as total species richness in models of the 2011 and combined data. Phosphorus-driven gradients were only identified as a predictor of *A. stamineus* density and *S. stimpsoni* density in models of the 2011 data. Though Poeciliid density was a contributing driver of *A. stamineus* density as well as native, invasive and total species richness, its predictive power was highly variable (Table 10). *A. stamineus* population density was only identified as a predictor of native species richness in models of the 2011 data.

Comparison of relationships among islands: Some of the inconsistencies among the stepwise regression model results are likely attributable to relationships varying among islands for a particular year and/or spatial scale. Variation in response to land use (where land use is considered a categorical condition based on whether forest or ag-urban is the prevailing land cover for sites and watersheds) serves an example of how relationships between biotic and abiotic factors can differ among islands. Though some trends suggest otherwise (Figure 54), responses of *A. stamineus* and *S. stimpsoni* genetic diversity measures and total species richness to land use did not significantly differ among islands in either year or spatial scale (ANOVA, $p > 0.05$). Responses of *A. stamineus* density and Poeciliid density to land use did not significantly

differ by island (ANOVA, $p > 0.05$) in 2009, although a statistically significant signature of island-level variation in *A. stamineus* density responses was detected among sites in the 2011(ANOVA, $p = 0.05$) and combined (ANOVA, $p = 0.012$) data sets, and for Poeciliid density among watersheds (ANOVA, $p = 0.05$) and sites (ANOVA, $p = 0.004$) in the 2011 data. Significant island-level variation in response to land use was consistently detected for *S. stimpsoni* density, goby density, native species richness, and invasive species richness among years and spatial scales (ANOVA, all $p < 0.05$), with Molokai or Oahu having the greatest (and generally opposing) effect sizes (Figure 55).

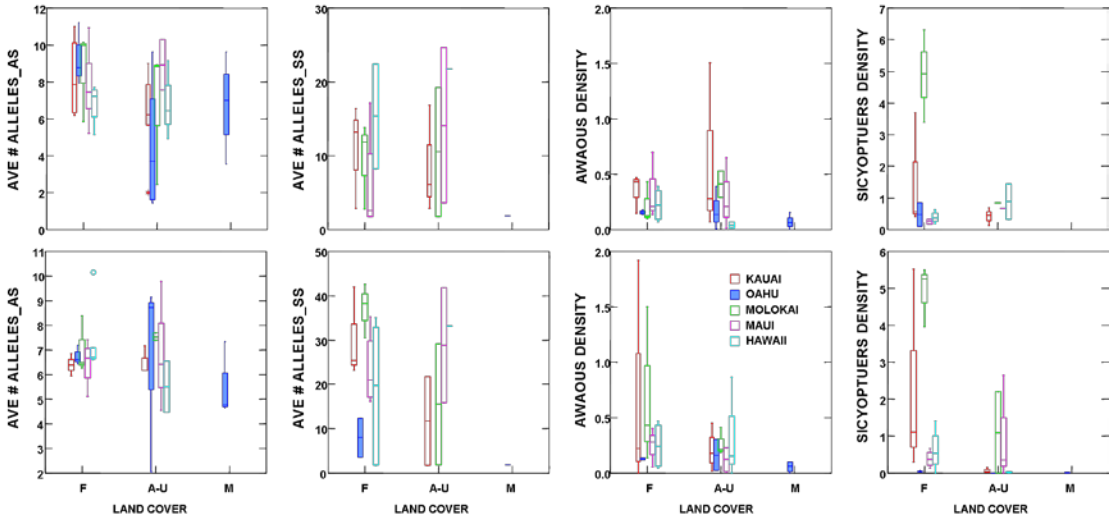

Figure 54: Comparison of representative measures of genetic diversity and population density for *Awaous stamineus* (AS, Awaous) and *Sicyopterus stimpsoni* (SS, Sicyopterus) in 2009 (top) and 2011 (bottom) among watersheds across islands, with specific reference to Oahu, according to dominant land cover (F = forest, A-U = ag-urban) and stewardship (M = military). ANOVA values are provided in the text.

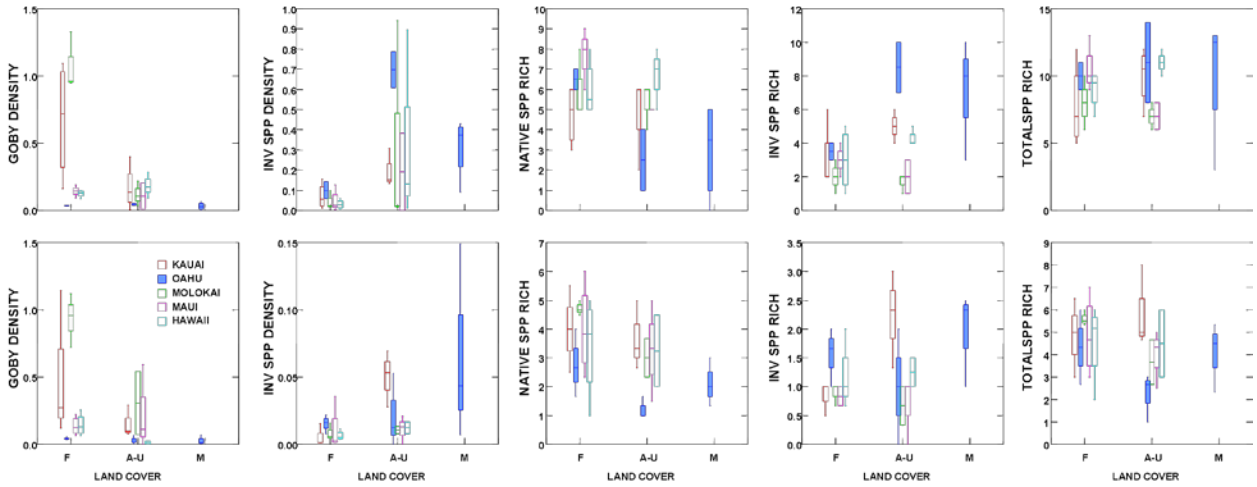

Figure 55: Comparison of representative measures of population density for native gobies and invasive species, as well as native, invasive and total species richness in 2009 (top) and 2011 (bottom) among watersheds across islands, with specific reference to Oahu, according to dominant land cover (F = forest, A-U = ag-urban) and stewardship (M = military). ANOVA values are provided in the text.

Comparison of areas under military stewardship to other watersheds on Oahu: Categorical comparisons among sites and watersheds on Oahu indicate that areas under military stewardship harbor population densities and levels of diversity comparable to other watersheds on Oahu.

Though areas under military stewardship are predominantly forested (>50% total), some watersheds are heavily urbanized or have been converted to agricultural landscapes. Accordingly, population densities and measures of genetic and species diversity often ranged between those found in predominantly forested and ag-urban watersheds (Figure 56).The exceptions that were observed did not indicate that watersheds under military stewardship were more impaired than other watersheds on Oahu. In comparisons restricted to 2009 data, significantly higher invasive species densities were detected in ag-urban watersheds across both watershed- (ANOVA, $p = 0.018$) and site-level (ANOVA, $p < 0.001$) comparisons. Poeciliid densities were also higher in ag-urban watersheds (ANOVA, $p < 0.001$) in site-level comparisons. Shannon diversity of alleles (ANOVA, $p = 0.047$) was significantly higher in military watersheds than ag-urban watersheds, and total species richness was higher in military watersheds than forested watersheds (ANOVA, $p = 0.025$).

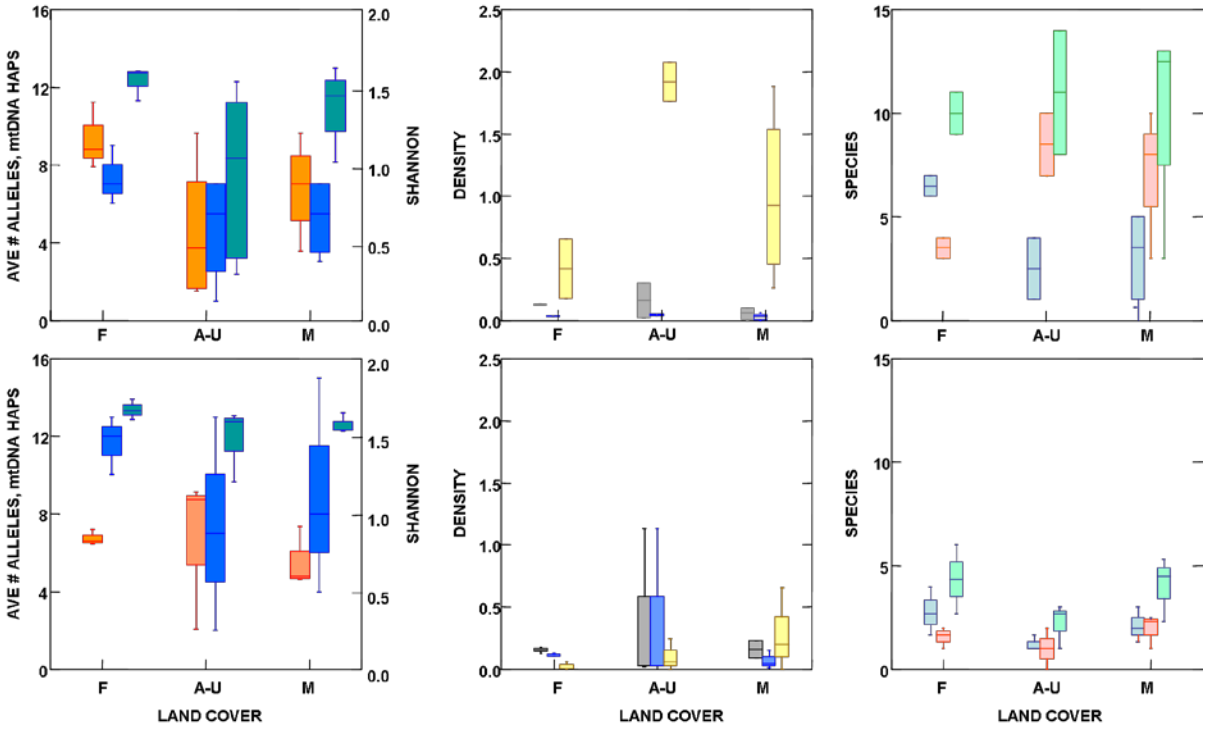

Figure 56: Comparison of *A. stamineus* genetic diversity (left; Shannon = green, NAA = orange, mtDNA = blue), *A. stamineus* population density (middle, grey), total goby density (middle, blue), Poeciliid density (middle, yellow), native species richness (right, light blue), invasive species richness (right, pink), and total species richness (right, light green) in 2009 (top) and 2011 (bottom) among watersheds on Oahu according to dominant land cover (F = forest, A-U = ag-urban) and stewardship (M = military). ANOVA values are provided in the text.

Discussion: This study was undertaken to determine the extent to which the well-being and integrity of at-risk native Hawaiian stream fishes- measured in terms of genetic diversity, population densities and species richness- vary in response to biotic and abiotic stress. This involved comparing measures of genetic, population, and assemblage-level variation to in-stream conditions and watershed land use. We adopted a hierarchical sampling design to gain perspective on within-watershed, among-watershed, among-island, and temporal variance across areas dominated by forested and ag-urban land cover. We also examined areas under military

stewardship (i.e., watersheds used for military exercises or that are dominated by a DoD installation) on Oahu, which hosts a dense array of military installations. This approach afforded opportunities to evaluate stressor responses and indicator performance over multiple spatial scales and over time. Though clustering of military installations on Oahu prevented statistically valid comparisons of responses to military stewardship across islands, we were nonetheless able to determine whether areas under military stewardship differ from other areas across Oahu which provided critical information on island-wide outcomes of local degradation, as well as actions the military might carry out to promote the recovery of at-risk species.

Genes-to-ecosystems comparisons of native fish responses to alteration of landscape and in-stream conditions were carried out through a step-wise process of data collection and analysis. In addition to obtaining tissues from *A. stamineus* and *S. stimpsoni* for subsequent genotyping and sequencing, population densities of all native and non-native aquatic macrofauna (i.e., fish, molluscs, and shrimp) were surveyed at each study site. Fish assemblage structure, in-stream habitat conditions, and water chemistry also were characterized following standard protocols (Lazorchak 1998, Kido 2002). Land use within each watershed was characterized from satellite imagery (Blum et al. 2005, Blum et al. 2012), and nitrogen stable isotope ratios of algae, snails, and *A. stamineus* were used as a time-integrated measure of anthropogenic nutrient loading (Fry 1999, Schlacher 2005). The sensitivity of genetic, population, and assemblage indices to environmental variation was then evaluated through correlational comparisons, analysis of variance, and regression modeling (Blum et al. 2012). This enabled us to identify biotic and abiotic drivers of variation and to assess the condition of watersheds across the archipelago according to the status of resident populations of native fishes.

Within and among island comparisons indicate that the integrity of native fish populations declines with increasing densities of invasive species and intensification of land use (ie. from forested to heavily urbanized), and that native fish populations occupying areas under military stewardship are comparable to populations elsewhere on Oahu. Basic physiographic features that control carrying capacity (e.g., watershed area) and habitat availability (e.g., distance to the stream mouth) appear to be primary determinants of genetic diversity, population densities and native species diversity. Invasive species and land use intensification, on the other hand, appear to diminish densities and diversity of native species. Our findings also indicate that biotic and abiotic degradation varies among islands, with watersheds on the windward shore of Molokai supporting many of the densest populations of native fishes, and watersheds on Oahu hosting the highest densities and diversity of non-native species. We also found evidence that densities of native fishes are depressed across Oahu. Categorical comparisons of watersheds across the archipelago showed that forested watersheds harbor higher densities of native fish populations, except on Oahu. Though predominantly forested watersheds support greater native species richness and more genetically diverse populations of native fishes (Figure 56), low population densities of our target species and of all gobies were found across Oahu. The density of populations occupying areas under military stewardship did not deviate from this pattern-densities of these populations fell well within the range observed for populations in other watersheds on Oahu. One of our target species, *S. stimpsoni*, was rarely encountered on the island. Though densities of *A. stamineus* were not statistically different from densities observed on other islands, maximum densities on Oahu were consistently among the lowest observed across the archipelago. These findings suggest that aggregate effects can arise from local and

watershed-scale degradation, where the cumulative influence of biotic or abiotic stressors can disrupt processes that promote the persistence of native fishes across entire islands.

A primary objective of this study was to evaluate whether measures of genetic diversity in native migratory fishes reflect physiographic and demographic conditions. This involved examining patterns of genetic variation exhibited by *Sicyopterus stimpsoni*, an endemic, moderately intolerant species capable of dispersing far inland, as well as patterns exhibited by *Awaous stamineus*, which is a more tolerant putatively endemic species common to lower and middle elevation stream reaches (Keith 2003, Lindstrom et al. 2012). Our findings indicate that variation in genetic diversity is partly attributable to both physiographic and demographic conditions. For both species, we found that population densities were a primary driver of genetic diversity, which is consistent with what has been found for other fishes, including non-migratory species (Blum et al. 2012). When population densities were excluded from consideration, physiographic controls of carrying capacity and habitat availability were consistently identified as predictors of genetic variation. This suggests that, without any additional influences, larger watersheds harbor larger and more genetically diverse populations of native stream fishes. Genetic diversity also corresponded to longitudinal position within watersheds, with the effect likely contingent on accessibility (i.e., to immigrating postlarvae). Genetic diversity in *A. stamineus*, which generally cannot access higher inland reaches, declines with increasing distance from the stream mouth. Similar longitudinal patterns have been found in other benthic stream fishes, though patterns in other species are attributable to different underlying processes such as historical colonization of watersheds and low movement potential (Hänfling and Weetman 2006, Blum et al. 2012, Lamphere and Blum 2012). Genetic diversity in *S. stimpsoni*, on the other hand, increases with increasing distance from the stream mouth. This suggests that, barring the influence of other factors, more genetically diverse *S. stimpsoni* populations would be expected at higher inland reaches in larger watersheds. However, observed levels of genetic diversity and population densities often deviated from expectations based solely on physiographic conditions.

Another primary objective of this study was to evaluate the extent to which measures of genetic diversity in native migratory fishes vary according to in-stream and watershed conditions. It is well understood that increasing environmental impairment in aquatic ecosystems can lead to monotonic declines in species diversity, reflecting reductions in population size and loss of intolerant species (Karr 1981, Hughes and Noss 1992, Bagley et al. 2004). Environmental impairment can also lead to monotonic declines in genetic diversity of stream fishes (Blum et al. 2012), but interactions between historical and contemporary factors can result in complex patterns of variation within and among oceanic islands. The sequence and timing of initial colonization can give rise to differences among islands, with the highest levels of genetic diversity occurring on the island(s) harboring the original, founding population(s). Following stepping-stone colonization of other islands, the most recently derived population would be expected to exhibit the lowest levels of genetic diversity (see section 5.0.1). However, deviations from this pattern may occur because coarse scale biogeographic filters, such as island age, may constrain or amplify the expression of biotic or abiotic stressors at finer spatial scales (Poff 1997). For example, both watershed geomorphology (e.g., slope) and geophysical characteristics of soils vary with island age (Vitousek 2004). Consequently, sediment transport to streams also may differ according to island age, where concentrations of suspended or dissolved sediments might be expected to be higher in streams on Kauai, as would the extent to which land use

intensification promotes sediment transport to streams. Levels of genetic diversity therefore may be lower than expected in watersheds on Kauai where native fish populations are subject to coarse scale filtration of contemporary stressor conditions. Impairment may also independently result in deviations from expected levels of diversity, including diversity gradients established by historical colonization. If immigrants are in short supply, local and watershed-scale impairment can depress the density and diversity of native fish populations. A continuous influx of immigrants driven by heavy propagule pressure, on the other hand, may sustain expected levels of genetic diversity and possibly give rise to positive relationships between genetic diversity and measures of impairment.

With some important exceptions, our results indicate that the genetic diversity of native Hawaiian stream fishes declines with environmental impairment. Pair-wise correlations and regression models indicate that genetic diversity within populations of native fishes is consistently inversely related with greater agricultural and urban land use within a watershed and corresponding in-stream conditions associated with nutrient loading and sedimentation. These findings underscore conclusions from prior studies that native Hawaiian stream fishes are sensitive to in-stream and watershed conditions attributable to land use intensification (Brasher 2003, Brasher and Wolff 2004, Brasher et al. 2006). Our results also highlight that native stream fishes may be particularly sensitive to intensification of lower watershed regions (i.e., estuaries and stream mouths). All individuals within a watershed experience lower watershed regions as residents, emigrating larvae, or immigrating postlarvae. Consequently, the influence of land use intensification around estuaries or stream mouths can manifest across an entire watershed. Impervious surfaces in lower watershed regions could, for example, increase exposure of all individuals to contaminants carried in run off. By changing the chemical composition of freshwater plumes (which may serve to attract marine dispersing immigrants), impervious surfaces in lower watershed regions might also depress population densities throughout a watershed by reducing re-entry of postlarvae to the stream. We also found that genetic diversity declines with increasing densities of non-native species, which is consistent with other studies that have linked the reduction of goby population densities to direct and indirect interactions with invasive species (Brasher et al. 2006, Holitski et al. 2013). As with land use, we uncovered indications that invasive species can influence native stream fishes throughout a watershed. Prevalent invasive species like Poeciliids can directly impact co-occurring native fishes through resource competition and by spurring shifts in nutrient and habitat availability (Holitski et al. 2013). It is also possible that Poeciliids and other invasive species (e.g., cichlids and bass) reduce larval export and postlarval immigration through predation (Holitski et al. 2013, Hain et al., unpublished data). If so, then predatory invasive species occupying lower watershed reaches represent biological gauntlets that may limit population densities of native fishes throughout a watershed.

The strength of environment-driven changes in genetic diversity can vary among species according to life history or tolerance to environmental impairment, therefore cross-species comparisons are critical for understanding the nature of relationships between genetic diversity and stressors. The sensitivity, performance and value of genetic diversity (and related parameters) as a surrogate measure of aquatic environmental condition may depend on the choice of study organism. As in prior studies (Bagley et al. 2004, Blum et al. 2005, Blum et al. 2012), we examined a widely distributed native species that can be a dominant member of local

assemblages. Though *A. stamineus* achieves significantly higher population densities in uninhabited forested watersheds (e.g., on Molokai), the species is moderately tolerant and can therefore be found in a wide range of degraded or modified habitats. Unlike prior studies (Bagley et al. 2004, Blum et al. 2005, Waits et al. 2008, Blum et al. 2012), we also examined a moderately intolerant native species. Though *S. stimpsoni* can dominant stream fish assemblages, the species is rarely found in degraded watersheds. The difference in tolerance between the two species was evident in the observed relationships between measures of genetic diversity and impairment. For example, measures of genetic diversity in *S. stimpsoni* were consistently more sensitive to in-stream and watershed measures of increasing land use intensification. Similarly, measures of genetic diversity in *S. stimpsoni* were consistently more responsive to measures of biotic degradation, such as the density of non-native Poeciliids. These findings suggest that *S. stimpsoni* is a more revealing sentinel than *A. stamineus*, and that monitoring programs should selectively favor it over *A. stamineus* or other possible alternatives (i.e., other native fish or invertebrates). This might prove sensible in many regions of the Hawaiian archipelago where the species is still common and abundant, but it would provide limited capacity to assess and track conditions on Oahu, where the species is rare or absent in watersheds across the island. Thus relying on *S. stimpsoni* as a sentinel would be of limited value to DoD water quality and watershed assessment programs. Similarly, relying solely on *S. stimpsoni* would be of limited value for monitoring urban or urbanizing areas on other islands, such as watersheds surrounding Lihue on Kauai and Kahului on Maui. Our findings indicate that approaches involving cross-species comparisons can capture a wider range of environmental conditions and provide more comprehensive perspectives on diversity in Pacific island stream ecosystems which in turn could improve diagnosis of anthropogenic influences on aquatic environmental condition (Noss 1990, Bagley et al. 2004, Blum et al. 2012). Our findings also suggest that assessments focusing on more tolerant species, like *A. stamineus*, can be useful for capturing early responses to actions taken to improve stream conditions (i.e., tolerant species are likely to be pioneering species), particularly on Oahu where smaller propagule pools of less tolerant species may limit recolonization.

Among-watershed comparisons indicate that genetic diversity, population densities and assemblage structure of native stream fishes all decline with biotic and abiotic impairment. Evidence of parallel responses to impairment might suggest that measures of genetic diversity do not necessarily improve or enhance understanding of stream condition on Pacific islands. However, information on genetic variation provides distinct and valuable perspectives on responses to impairment and processes that sustain populations in degraded waterways (Waits et al. 2008). Because information on genetic variation can complement information on population densities and species diversity, genetic assessments may help overcome limitations of traditional assessment approaches. All protocols currently used to assess oceanic island streams rely on population or assemblage-level measures to rate the condition of stream segments. Protocols implemented in the Hawaiian Islands (e.g., Burr 2001, Kido 2002, Henderson 2003), which are largely modifications of protocols for continental stream ecosystems (e.g., Lazorchak et al. 1998), involve measuring the diversity and relative abundance of native fishes. Though these measures reflect the conservation importance of Hawaiian stream biota, the approach offers little basis for discriminating between levels of impairment in areas most in need of management and restoration. Conditions across Oahu illustrate the limitations of assessments based on population densities and species diversity. Cross-archipelago comparisons show that densities of native

fishes are depressed on Oahu (Figure 55), and categorical comparisons of watersheds on Oahu indicate that population densities of native fishes are similar regardless of prevailing land use conditions (Figure 56). Population densities of native fishes in forested watersheds were comparable to densities of native fishes in urban watersheds. Though forested watersheds on Oahu harbored more native fish species (Figure 55), the information content of ratings based on assemblage-level characteristics is minimal (Parham 2005) because the native fish assemblage is naturally depauperate. Ratings of condition might therefore differ according to small, incremental changes in species richness (i.e., the presence of two native species versus one or no species being present). Assemblages of native species on Oahu also were typically dominated by those capable of tolerating impaired conditions, such as *A. stamineus* and *Eleotris sandwicensis*. Intolerant species, such as *S. stimpsoni*, *Lentipes concolor*, and *Neritina granosa* are exceedingly rare on the island. Consequently, ratings based on native species richness may prove to be even less informative even with incremental differences among sites or watersheds. Reliance on measures of population density or species diversity could also bias assessments towards underestimating impairment. For example, estimates of population density or species diversity would fail to accurately portray impairment if populations are being sustained by immigration of postlarvae originating from other streams. Measures of genetic diversity, on the other hand, may offer a stronger basis for detecting and rating impairment on Oahu. Genetic diversity of native fishes is not as severely depressed as population densities (Figures 55 and 56), which is consistent with theoretical expectations that the loss of genetic diversity lags behind demographic decline (Spielman et al. 2004). Differences in genetic variation, either among locations or across time, also can capture the influence of demographic processes that are difficult to detect otherwise, including the magnitude and source of immigration (e.g., Lamphere and Blum 2012, Ferguson et al. 2013). Thus, integrative protocols that are tailored to capture estimates of genetic variation alongside measures of population density and species diversity could offer a stronger basis for management and restoration of streams and conservation of constituent biota on Oahu and elsewhere.

A combination of approaches also can prove valuable because discordant patterns of genetic variation, population densities and species diversity can provide information that might not otherwise come to light (e.g., Lamphere and Blum 2012). Environmental impairment, for example, may not necessarily lead to parallel declines in genetic diversity and species diversity. Genetic diversity is expected to fall with decreasing population size (Frankham 1996), but declining population sizes of a dominant species might increase species diversity if the species is replaced by others in an assemblage (Vellend 2005, Blum et al. 2012). An inverse relationship between genetic diversity and species diversity might occur if replacement results in resource competition that reinforces responses of the declining species to abiotic impairment. On Oahu and across the archipelago, we consistently detected positive relationships between genetic diversity and native species diversity. Negative relationships were detected between genetic diversity in *S. stimpsoni* and invasive species richness across both 2009 and 2011 (watersheds; Shannon, $r = -0.362$, $p = 0.05$; NAA, $r = -0.410$, $p = 0.027$), though no relationships were recovered for measures of genetic diversity in *A. stamineus* and invasive species richness (all, $p > 0.05$). This suggests that *S. stimpsoni* do not shoulder the added burden of interactions with invasive species as well as *A. stamineus*. The absence of relationships between genetic diversity and total species richness, a measure that accounts for both native and non-native species (all, p

> 0.05), also indicates that non-native species are largely replacing rather than co-existing with native species (Figure 50).

Integrative characterization of conditions on Oahu provides another example of how discrepancies can offer novel perspectives on impairment that can help DoD and other land stewards identify management strategies that are more likely to yield desired outcomes (i.e., large, stable populations of native species). By itself, evidence of depressed population densities across Oahu suggests that widespread impairment of in-stream conditions is lowering survival of native stream fishes (residents and immigrants alike). Managers acting on this interpretation might choose to allocate resources to improve local habitat conditions to increase successful immigration and the persistence of local populations. Additional evidence of low levels of genetic differentiation and modest reductions in genetic diversity indicates that widespread degradation on Oahu may also be dampening propagule pressure across the island. Evidence of weak genetic differentiation and modest reductions in genetic diversity suggests that the pool of immigrants is principally derived from contributions of populations on Oahu. Weak genetic differentiation (i.e., high gene flow) indicates that exchange among watersheds on Oahu is highly likely. Though weak genetic differentiation might also suggest extensive exchange occurs among islands, modest reductions of genetic diversity indicates that immigration from other islands is not sufficient to compensate for the reduced productivity of impaired populations on Oahu. Without compensatory propagule pressure originating from other islands, sparse populations of native fishes would be likely to occur across Oahu, even in watersheds that are not impaired. Observed densities in remote forested watersheds on Oahu are consistent with this expectation. Managers acting on this inference might implement strategies to not only improve local habitat conditions, but to also enhance propagule pressure by augmenting key populations that could serve as sources of immigrants to watersheds across Oahu.

Corroborative evidence from independent investigations provides further support for management strategies that aim to improve local conditions and regional scale processes. Our study of otolith microchemistry supports the inference that immigrant pools are predominantly composed of individuals derived from local watersheds (i.e., on one island rather than from islands across the archipelago). We found that the majority of *A. stamineus* larvae do not enter the marine environment (see section 5.0.3), and that larvae entering the marine environment often occupy near-shore rather than off-shore environments (i.e., thus increasing the likelihood of local retention). Coupled biophysical model simulations (see section 5.0.5) also indicate that the probability of marine dispersing larvae re-entering streams is exceedingly low (i.e., often <5%), which suggests that cross-island contributions to immigrant pools is small. Model simulations further indicate that the probability of local retention can be high, particularly when larval export is scaled to river discharge (see text below).

Our effort to reconstruct hierarchical genes-to-ecosystem patterns offers unprecedented opportunities to evaluate contemporary watershed conditions across the Hawaiian archipelago. No prior assessments have examined watersheds on all islands that support perennial waterways. Prior assessments have either focused on a single watershed (e.g., Burr 2001, Englund et al. 2001, Henderson 2003, Englund and Arakaki 2004) or a set of watersheds in a region of one island (e.g., Brasher et al. 2004, Gingerich and Wolff 2005, Wong 2005), though three independent efforts have been undertaken to evaluate larger scale patterns of watershed condition

across the State. Through the NAWQA program, the USGS examined water quality and biotic integrity of streams across Oahu (Anthony et al. 2004). A complementary pilot study conducted by the USGS and Hawaii Department of Health (DoH) similarly examined water quality and biotic integrity of streams across Oahu (Wolff and Koch 2009). And, the Hawaii DAR has prepared the Hawaii Watershed Atlas (Hawaii Watershed Atlas; http://hawaii. gov/dlnr/dar), which includes compilations of historical biotic survey records and corresponding ratings of all watersheds across the State. Like the study presented here, the USGS NAWQA study (Anthony et al. 2004) considered a suite of physical, chemical and biological parameters to assess aquatic environmental condition. The NAWQA assessments, which were completed in 1999-2001, were based on extensive sampling (i.e., sites in a dozen or more watersheds) and categorical comparisons of parameters to understand the nature of stream impairment. The pilot study completed by Wolff and Koch (2009) involved a similar study design, though assessments of condition focused on benthic macroinvertebrate assemblage structure. Neither study included comparisons to watersheds on other islands, therefore neither provided a context for understanding conditions on Oahu. The studies also do not provide sufficiently explicit comparisons to offer information on the condition of areas under military stewardship. The Hawaii Watershed Atlas offers an exceptional amount of biotic and landscape-level information on watersheds throughout the archipelago, but the anthology was not explicitly designed for the purposes of assessing aquatic environmental condition. For example, no information is provided on in-stream habitat conditions or water chemistry. Information presented on biotic diversity (i.e., the presence of native and invasive species) also is an amalgamation of data from all available records (i.e., from multiple sources), which does not offer a basis for comparing biotic diversity among watersheds at a specific time or across specific time intervals. Consequently, the watershed ratings are not a reflection of contemporary or baseline conditions. The ratings do offer a context for interpreting time-specific assessments of stream condition. For example, new biotic survey records can be compared to the assembled records to determine whether particular species of interest have previously been documented in a watershed or region of a watershed (i.e., low, middle, or high elevation reaches). Thus our work not only represents the first explicitly contemporaneous survey of watershed conditions across the State, it also offers a basis for comparing areas under military stewardship to other areas across Oahu and other islands.

Several important conclusions can be drawn from cross-island comparisons about the prevalence and distribution of impaired watersheds in Hawaii. First, many of the most impaired watersheds in the State occur on Oahu. Oahu supports some of the lowest densities of native fishes, the highest densities of non-native fishes, and the highest proportions of impervious surfaces in watersheds across the archipelago. Similarly, many the most nutrient-laden stream reaches occur on Oahu. It is noteworthy that severely impaired watersheds also occur on other islands. Urban and urbanizing watersheds surrounding Lihue on Kauai harbor sparse populations of native fishes. Urban watersheds surrounding Kahului on Maui, including Iao watershed (the site of an important state and cultural park), also harbor sparse populations of native fishes. Though watersheds with predominantly ag-urban land cover generally supported smaller, less genetically diverse populations of native fishes, comparison of biologically impaired watersheds across Oahu, Kauai, and Maui demonstrates that different factors can reduce population densities of native fishes. Aggregate effects of local and watershed-scale degradation are likely depressing native fish populations across Oahu, whereas low densities of native fishes in middle and upper elevation reaches of some of the largest watersheds on Kauai (e.g., Wailua River, Hanamaulu

River) are likely a consequence of predation by non-native sport fishes (e.g., large-mouth and small-mouth bass) introduced to lower and middle elevation reaches. Low densities of native fishes in Iao watershed on Maui, on the other hand, are largely attributable to water abstraction. Iao River, which is a major source of water for residential and agricultural use on Maui, is heavily diverted and rarely connects with the ocean. Indeed, it is unclear whether infrequent connection to the ocean is sufficient to supply immigrants originating from the watershed or elsewhere. Many, and possibly all, of the native amphidromous fishes and invertebrates that occupy Iao River above the diversion have been transplanted from other watersheds by the DAR (S. Hau, personal communication).

Several important conclusions also can be drawn from cross-island comparisons about the prevalence and distribution of unimpaired watersheds in Hawaii. Remote, forested watersheds occur on every island in the State with perennial waterways (Niihau, Lanai, and Kahoolawe do not presently support perennial waterways). However, the biological integrity of these watersheds varies considerably. For example, attributes of native fish populations in remote forested watersheds on Oahu, such as Kahana watershed (the site of a state park) on the northwestern windward coast, are similar to those of populations in urban and urbanizing watersheds elsewhere on the island. Forested watersheds on Kauai and Molokai, on the other hand, support many of the densest and genetically diverse native fish populations in the archipelago. Unlike most of the forested watersheds on Oahu, many of the watersheds harboring dense and diverse populations on Kauai and Molokai are not readily accessible or have little to no human habitation. For example, Hanakapiai watershed on Kauai's Napali Coast (a state park) is only accessible by boat or foot. Pelekunu watershed (a nature preserve managed by The Nature Conservancy) on the windward coast of Molokai is only accessible by boat during periods of the year when off-shore winds are weak. Historical records also suggest that Pelekunu watershed has had little to no human habitation. Our findings indicate that remote forested watersheds on Kauai and Molokai could serve as universal references for monitoring and assessment programs across the State, particularly for watersheds on Oahu that exhibit similar physiographic features. Reference watersheds should also be identified on each island, however, because physiographic variation within and among islands can limit the significance of universal references. For example, a reference watershed would be valuable for the Hamakua coast of Hawaii (northwest of Hilo), where terminal waterfalls often constrain the diversity and densities of native fishes.

Prior assessments of watersheds on Oahu have demonstrated that some areas under military stewardship are severely impaired (e.g., Englund 1998, USAF 2000, Henderson 2003) but no systematic comparisons have been carried out to identify whether impairment is solely attributable to military activity, or whether impairment is partly attributable to other activity in watersheds with a military presence, or factors influencing all watersheds on Oahu. Unlike prior assessment, the design of this study enables comparisons to be drawn within and among watersheds on Oahu as well as among watersheds across islands. Categorical comparisons among sites and watersheds on Oahu indicate that areas under military stewardship harbor population densities and levels of diversity comparable to other watersheds on Oahu, with patterns of variation suggesting that the condition of areas under military stewardship largely reflect underlying land use. Though these watersheds are predominantly forested (>50% total coverage), particularly in middle to higher elevation regions, some sections are heavily urbanized or have been converted to agricultural landscapes. With few exceptions, the observed population

densities of native fishes and measures of genetic and species diversity fell within the range observed for populations in predominantly forested and ag-urban watersheds. Some of the most impaired sites on Oahu did occur in watersheds that fall under military stewardship. Sites in the lower region of Waimanalo watershed, for example, exhibited acutely elevated levels of nutrient loading. Conditions found in Waimanalo watershed, however, are not necessarily attributable to military activity. Two potentially confounding factors must be taken into consideration. First, impairment of lower watershed regions can result from effects arising from natural processes or non-military anthropogenic activities elsewhere in the watershed (ie. upstream from a DoD installation). Though multiple sites were examined in Waimanalo watershed for this study, sites were not selected to determine potential sources of impairment. More comprehensive longitudinal comparisons will be necessary to determine sources of impairment in Waimanalo. Signatures of impairment in Waimanalo watershed and elsewhere may also be partly attributable to widespread degradation dampening propagule pressure across the island. Inadequate propagule pressure could give rise to sparse populations of native fishes across Oahu, even in watersheds that are not otherwise impaired. Though forested watersheds on Oahu generally support greater native species richness and more genetically diverse populations of native fishes, comparisons to other islands demonstrated that the density and diversity of native fishes were depressed across Oahu. The condition of populations occupying areas under military stewardship did not deviate from this pattern, highlighting the possibility that assessments based on a single scale of observation might not capture the full range of responses to environmental conditions and therefore may yield misleading impressions of degradation and drivers of impairment.

5.1.3 Sensitivity of Genetic, Population, & Community Metrics to Within-Watershed Environmental Variation

Prevalence of study species, mark-recapture sampling, and tissue collections: Higher densities of *A. stamineus* were found at all study sites on Hawaii compared to sites on Oahu. Approximately 2,850 individuals were sighted or marked at the selected suite of sites over the course of the study (Table 4), with per-site total marks ranging from 13 to 294. Excluding sites in Waimea watershed on Oahu, the minimum per-site total marks was 71. Per-site total recaptures ranged from 4 to 260. Excluding sites in Waimea, the minimum recapture was 70 (Table 4). Tissue samples were obtained from 10 to 51 individuals at genetics-only sites. Between 13 and 201 individual genetic samples were collected at mark-recapture sites. In all, tissues were collected from 1,354 individuals from 22 sites over the course of the study (Table 13).

Water chemistry: There was no clear pattern of NH_4 values among the three watersheds on Hawaii. However, average values of NO_3 and SRP were moderately lower (all, $p > 0.05$) in Hanawi (7.56 and 1.74 respectively) than Maili (9.09 and 2.36 respectively), and considerably higher (all, $p < 0.05$) in Hiilawe (790.1 and 67.93 respectively). The differences are sufficiently large between Hiilawe and the other two watersheds to possibly result in a cascade of trophic differences including greater primary productivity and higher fish growth rates in Hiilawe. Average values of NO_3 and SRP observed in Waimea (2.82 and 2.53 respectively) were lower than those observed in Hanawi ($p < 0.05$) and Maili ($p > 0.05$). Average values of NH_4 were similar to those observed in all three watersheds on Hawaii (all, $p > 0.05$).

Estimates of population densities: Densities of *A. stamineus* were significantly lower in Waimea watershed than in the three watersheds on Hawaii (all, $p = 0.001$; Table 13). Average *A. stamineus* densities were highest in Maili watershed (survey mean = 0.3791, range = 0.0899-0.7643; m-r mean = 0.3955, range = 0.3368-0.4961) and lowest in Waimea watershed (survey mean = 0.0692, range = 0.05-0.0909; m-r mean = 0.0212, range = 0.0165-0.0282). Densities of *A. stamineus* were only marginally higher ($p = 0.708$) in Hiilawe (survey mean = 0.2586, range = 0.0938-0.6557; m-r mean = 0.2608, range = 0.1076-0.4118) than in Hanawi (survey mean = 0.2117, range = 0.0385-0.6207; m-r mean = 0.1703, range = 0.0857-0.3195).

Total goby density, derived from survey estimates, was highest in Hanawi watershed (mean = 0.8841, range = 0.25-1.7724), and lowest in Waimea watershed (mean = 0.1707, range = 0.1167-0.25). Maili watershed (mean = 0.8145, range = 0.125-1.6333) had marginally higher ($p = 0.2561$) total goby densities than Hiilawe watershed (mean = 0.4843, range = 0.1875-0.9359). Of the three watersheds with Poeciliids present (i.e., no Poeciliids were detected in Hanawi), survey estimates of Poeciliid densities were significantly higher (all, $p < 0.01$) in Maili watershed (mean = 1.3108, range = 0.0667-2.4514), where they were present at every site. Estimates were lower (all, $p < 0.01$) in Hiilawe watershed (mean = 0.0395, range = 0-0.2372) than other watersheds, where they were present at only one site. In Waimea watershed, Poeciliids (mean = 0.5129, range = 0-1.5273) were present at 3 of the 4 sites (Table 13). No Poeciliids were detected by visual surveying at the lowest site, though it is likely that they were present at low densities (Table 13).

Mark-recapture estimates of individual movement: Although movement of individuals between sites within a watershed was possible, only a few individuals were recaptured at a different location from their release site. The individual mark-recapture (IMR) study documented that only 28 individual *A. stamineus* (16 in Hanawi, 2 in Hiilawe, 10 in Maili) moved between sites. Of these, 24 moved downstream in the watershed, and 4 moved upstream in the watershed (2 in Hiilawe, 2 in Maili). The 4 that moved upstream were all under 40 mm when first marked. Of the 24 that moved downstream, only 3 were below 40 mm when first marked. The remainder ranged from 46 mm to 155 mm. Of the individuals that moved upstream in the watershed, the average distance traveled was 0.7462 km (range = 0.1828-2.13 km) and the average time span over which movement was detected was 3.25 months (range = 2-4 months). Of the individuals that moved downstream in the watershed, the average distance traveled was 0.4460 km (range = 0.1011-0.6528 km), and the average time span over which movement was detected was 2.875 months (range = 1-6 months).

Mark-recapture estimates of demographic parameters: Because of the low number of individual fish marked and recaptured in Waimea watershed (Table 4), all mark-recapture estimates of demographic parameters were limited to sites in Hiilawe, Hanawi, and Maili watersheds. The best fitting POPAN model allowed for seasonal variation in capture probability (*p*) and survival (*phi*), and individual estimates of the probability for entry into the population

Watershed	Site	Sample Type	Month	Latitude (W)	Longitude (N)	Distance to Mouth (km)	Poeciliid Presence	*N*	TL (MIN)	TL (MAX)	IMR Density	IMR SE	VS_Density	VS_SE	Skew	Kurtosis	Poeciliid Density	Goby Density
Hiilawe																		
	1	M-R	June-Nov	20.10716	155.59639	1.55	Y	57	27	152	0.1076	0.0186	0.3269	0.0582	1.4522	4.8066	0.2372	0.9359
	2	Genetics Only	Sept	20.1054	155.59573	1.75	N	49	23	91			0.0938	0.0679	0.0000		0.0000	0.1875
	3	M-R	June-Nov	20.10434	155.59578	1.89	N	201	25	187	0.4118	0.0359	0.6557	0.1117	1.6421	4.0795	0.0000	0.8279
	4	Genetics Only	Sept	20.1023	155.59451	2.19	N	26	27	118			0.1111	0.0655	0.0000	-1.2000	0.0000	0.2778
	5	M-R	June-Nov	20.09957	155.595	2.49	N	101	27	135	0.2630	0.0363	0.1875	0.0362	0.7282	0.8810	0.0000	0.3828
	6	Genetics Only	Sept	20.09814	155.59619	2.65	N	36	28	95			0.1765	0.0775	0.8655	0.2007	0.0000	0.2941
Hanawi																		
	1	M-R	June-Nov	19.80502	155.09163	0.09	N	64	18	274	0.0857	0.0217	0.2083	0.0459	0.9481	0.4700	0.0000	0.5278
	2	Genetics Only	Sept	19.80456	155.09238	0.21	N	21	21	137			0.0385	0.0377			0.0000	0.5769
	3	M-R	June-Nov	19.80522	155.09358	0.42	N	72	21	176	0.1057	0.0190	0.1818	0.0412	2.0657	4.3749	0.0000	1.3712
	4	Genetics Only	Sept	19.8051	155.09492	0.57	N	20	23	100			0.0645	0.0441			0.0000	0.8065
	5	Genetics Only	Sept	19.80481	155.09578	0.64	N	28	22	141			0.1563	0.0779	1.5800	2.8043	0.0000	0.2500
	6	M-R	June-Nov	19.80572	155.09573	0.74	N	104	20	155	0.3195	0.0599	0.6207	0.0905	0.4543	0.5876	0.0000	1.7724
Maili																		
	1	Genetics Only	July	19.75603	155.09341	0.15	Y	11	98	146			0.1000	0.0548	-1.5971		0.0667	1.6333
	2	M-R	June-Nov	19.75524	155.09521	0.39	Y	198	22	162	0.4961	0.0741	0.7643	0.0993	0.7854	0.1529	1.1786	1.3143
	3	M-R	June-Nov	19.75397	155.09642	0.57	Y	149	20	167	0.3368	0.0668	0.4936	0.0598	0.8885	0.7322	1.8013	0.9808
	4	Genetics Only	July	19.75249	155.09808	0.79	Y	51	20	142			0.5000	0.1500	2.4561	7.0311	1.7000	0.5000
	5	Genetics Only	July/Aug	19.74762	155.11031	2.44	Y	32	31	145			0.0833	0.0564			0.6667	0.1250
	6	M-R	June-Nov	19.74825	155.11227	2.70	Y	132	40	149	0.3535	0.0653	0.3333	0.0491	0.5174	-0.5547	2.4514	0.3333
Waimea	1	M-R	July-Aug/Oct	21.63253	158.05067	1.74	N	13	41	173	0.0165	0.0085	0.0667	0.0322	-1.0024	0.9839	0.0000	0.2500
	2	M-R	July-Aug/Oct	21.6307	158.04599	2.29	Y	22	31	111	0.0189	0.0086	0.05	0.0281	1.2933		0.0667	0.1167
	3	Genetics Only	July	21.63064	158.04265	2.67	Y	10	29	52							0.7333	
	4	M-R	July-Sept	21.63052	158.03883	3.22	Y	40	40	124	0.0282	0.0203	0.0909	0.0388	-1.9245	3.6667	1.5273	0.1455

Table 13: Site information and estimates of *Awaous stamineus* population characteristics, including number of individuals sampled (N), minimum total length (TL, MIN), maximum total length (TL, MAX), individual mark-recapture population density estimates (IMR Density) and standard error (IMR SE), visual survey population density estimates (VS_Density) and standard error (VS_SE), Skew, Kurtosis, Poeciliid density, and total goby density. M-R = mark-recapture; Aug = August; Sept = September; Oct = October; Nov = November; W = west; = North; km = kilometer; Y = present; N = absent; TL is provided in mm; IMR and VS density estimates are provided in number of individual / M^2.

(*pent*) for each capture event. The POPAN model also provided estimates of population size (*N-hat*) for each capture event (White and Burnham 1999). Estimates of *N-hat*, averaged per site ranged from 41.62 (SE = 10.53) for site Hanawi-1 to 217.51 (SE = 32.48) for site Maili-2 (Table 13). For comparative purposes, estimates were converted to densities (N/m^2), thus transforming minimum and maximum estimates of averaged *N-hat* values to density values of 0.11 and 0.50 respectively (Table 13).

The best fit Pradel models allowed for seasonal differences in *p*, *phi*, and apparent recruitment (*f*; Table 14). Estimates of *phi* were similar during dry (0.77, SE = 0.04) and wet (0.77, SE = 0.06) seasons, while *f* was higher during the dry season (0.26, SE = 0.06) than the wet season (0.16, SE = 0.05). Estimates of *phi* were slightly higher in Maili (0.79, SE = 0.05) and Hanawi (0.79, SE = 0.06) than Hiilawe (0.73, 0.05). Estimates of *f* were higher in Hanawi (0.31, SE = 0.06) than Maili (0.16, SE = 0.05) or Hiilawe (0.17, SE = 0.05). Overall, estimates of *phi* did not differ among watersheds according to the presence of Poeciliids (Figure 57), but *f* was higher in Hanawi where Poeciliids were absent, relative to both Maili (where Poeciliids are prevalent throughout) and Hiilawe (where Poeciliids are present in the lower watershed).

Watershed	Site	Goby Richness	*Phi*	*f*	*K*	*L*∞	*He*	*Ap*	*Ar*	*Naa*	Shannon	*Neb*
Hiilawe												
	1	4	0.610	0.173	0.035	257.277	0.657	0.011	5.918	12.231	1.697	10.400
	2	3					0.619	0.012	5.518	10.385	1.579	15.300
	3	4	0.763	0.138	0.017	481.297	0.631	0.003	5.663	17.077	1.715	5.600
	4	2					0.642		5.757	9.077	1.596	
	5	2	0.803	0.160	0.041	195.884	0.646	0.007	5.766	13.308	1.697	13.600
	6	2					0.542		5.320	8.769	1.500	
Hanawi												
	1	4	0.729	0.346	0.035	205.692	0.618	0.009	5.545	11.615	1.616	31.400
	2	3					0.597	0.042	5.677	7.077	1.477	101.900
	3	4	0.801	0.262	0.020	255.257	0.661	0.007	5.635	12.000	1.633	18.800
	4	2					0.569		5.615	7.385	1.482	53.300
	5	2					0.604	0.038	5.686	8.692	1.578	24.200
	6	3	0.744	0.278			0.644	0.007	5.675	13.308	1.694	23.300
Maili												
	1	4					0.694	0.083	5.936	6.000	1.448	54.000
	2	2	0.861	0.144	0.089	142.854	0.640	0.004	5.668	16.385	1.709	9.500
	3	2	0.696	0.264	0.066	161.506	0.626	0.006	5.796	16.231	1.727	5.400
	4	1					0.620	0.010	5.486	10.077	1.593	12.100
	5	2					0.643	0.022	6.067	9.231	1.665	7.300
	6	1	0.835	0.096	0.022	240.454	0.646	0.007	5.870	16.154	1.772	3.300
Waimea	1	3	0.392	0.324			0.636		5.223	5.692	1.388	36.800
	2	3	0.492	0.329			0.599		5.319	6.231	1.474	29.100
	3	1					0.556	0.024	5.313	8.077	1.389	4.900
	4	3	0.738	0.041			0.635	0.013	5.334	9.385	1.534	23.700

Table 14: Site-specific estimates of native goby species richness, *Awaous stamineus* demographic characteristics, as well as estimates of genetic diversity and effective population size; *Phi* = survival, *f* = apparent recruitment, *K* = , *L*∞ = , *He* = expected heterozygosity, *Ap* = private allele frequency, *Ar* = rarified allelic richness, *Naa* = average number of alleles, Shannon = Shannon diversity of alleles, *Neb* = effective population size.

Growth coefficients developed from von Bertalanffy growth curves were lowest in Hiilawe (mean K = 0.031; Table 14), while the theoretical asymptotic maximum length estimate was highest (mean $L\infty$ = 312 mm; Table 14). Mirroring this finding, Maili had the highest estimates of K (0.058) and lowest estimates $L\infty$ (182 mm). Hanawi estimates were intermediate for both K (0.027) and $L\infty$ (231 mm; Table 14).

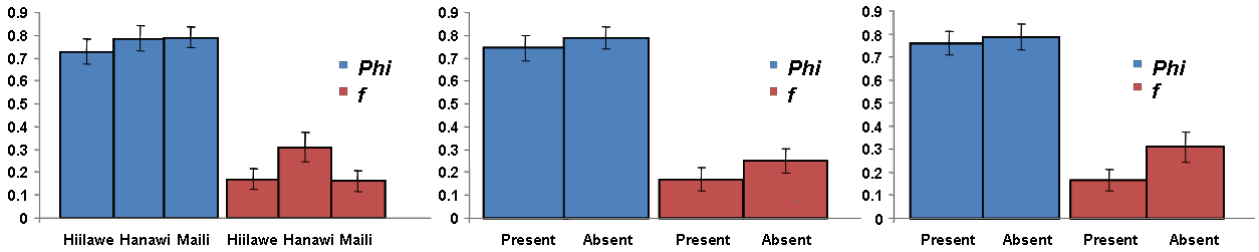

Figure 57: Comparison of demographic parameters among three watersheds on Hawaii (left), and according to the presence and absence of invasive Poeciliid fishes (mid, right). Statistical values are provided in the text.

Genetic diversity in Awaous: Estimates of observed heterozygosity were consistently high across study sites, with a mean observed heterozygosity of 0.6238 among sites (Table 14). Private alleles were observed in each of the four study watersheds, although five sites (Hiilawe-6, Hanawi-4, Hanawi-4, Waimea-1, and Waimea-2) contained none. A total of 72 individuals were found to possess private alleles at 1-3 separate loci. Private allele frequencies ranged from 0.0026 at *D3* in Hiilawe-3 to 0.0833 at *D110* in Maili-1.

Estimates of genetic diversity were lower in Waimea than in the watersheds on Hawaii. Estimates of the average number of alleles were significantly different between Waimea (mean = 7.346) and Maili (mean = 12.346), and estimates of Shannon diversity were significantly different in Waimea (mean = 1.4466) compared to Hiilawe (mean = 1.6307), Hanawi (mean = 1.5802), or Maili (mean = 1.6524). However, for watersheds on Hawaii, estimates of genetic diversity did not differ between watersheds, and were not strongly attributable to key physiographic conditions, such as longitudinal position within watersheds. Values of Shannon diversity, which ranged from 1.4485 at site Maili-1 to 1.7717 at site Maili-6, were similar among watersheds (p = 0.4394), with observed variation partly attributable to distance to the stream mouth (r^2 = 0.1079). Rarefied allelic richness was likewise similar between watersheds (p = 0.2280), though the variation had little to do with distance to the stream mouth (r^2 = 0.017).

Effective population sizes for *A. stamineus* on Hawaii ranged from 137.9 to 5705.4 individuals based on the linkage disequilibrium method. Estimates based on this method could only be estimated for one site in Waimea (Waimea-3, 92.7 individuals) due to low sample sizes. Non-infinite values could not be estimated for Hiilawe-6, Hanawi-2, or Maili-1 (Table 14).Values derived from the molecular co-ancestry method ranged from 3.3 to 101.9 individuals. Non-infinite values could not be estimated for Hiilawe-4 or Hiilawe-6 (Table 14). For both methods, sites on Hawaii where non-infinite values of effective population size could not be estimated were sites where mark-recapture studies were not carried out. These sites had, on average, smaller samples sizes (Table 13).

Genetic differentiation in Awaous: AMOVA results involving all sites indicated that no molecular variance is attributable to differences among watersheds (Table 15) or among sites within watersheds. Variance was attributable to differences among individuals. A temporal AMOVA indicated that 1.06% of molecular variance occurred among sites, with no variation attributable time (i.e., month within sites), and the remaining variation attributable to individuals

A

Variance component	d.f.	% variance	p-value
Among watersheds	2	0.05	1
Among sites within watersheds	15	-1.02	1
Within watersheds	2684	100.97	0.6501

B

Variance component	d.f.	% variance	p-value
Among watersheds	2	-0.07	1
Among age class within watersheds	3	-0.25	1
Within watersheds	2696	100.32	0.6735

C

Variance component	d.f.	% variance	p-value
Among watersheds	2	0.63	1
Among months within watersheds	15	-3.68	1
Within watershed	2684	103.05	0.1505

D

Variance component	d.f.	% variance	p-value
Among sites	17	1.06	1
Among months within sites	42	-0.1751	1
Within sites	2642	104.44	0.0489

E

Variance component	d.f.	% variance	p-value
Among months	5	-1.61	1
Among watersheds within months	12	-1.86	1
Within watersheds	2684	103.47	0.99511

F

Variance component	d.f.	% variance	p-value
Among High/Low	5	0.37	1
Among watersheds within High/Low	12	-0.86	1
Within watersheds	2684	100.49	0.08309

G

Variance component	d.f.	% variance	p-value
Among watersheds	3	-0.01	1
Among sites within watersheds	18	-0.85	1
Among individuals within sites	1414	-8.52	1
Within individuals	1436	109.39	0.77419

Table 15: Analysis of molecular variance (AMOVA) for microsatellite allelic variation among 3 watersheds on Hawaii (A-F), and for 4 watersheds on Hawaii and Oahu (G). High = high site(s) within watersheds; Low = low site(s) within watersheds. Negative values are indistinguishable from 0, and values > 100 are indistinguishable from 100.

within watersheds (Table 15). The temporal AMOVA only included watersheds on Hawaii because of limited sampling in Waimea over time. Restricting comparisons to sites on Hawaii increased the amount of molecular variance attributable to differences among sites and watersheds, but not by time (Table 15). Excluding sites in Waimea, 0.05% of molecular variance is attributable to differences among watersheds, and 1.02% is attributable to differences among sites within watersheds. This suggests that there are larger differences among watersheds on Hawaii, or that the distribution of variance may have been influenced by the relatively smaller samples sizes in Waimea watershed. It also suggests that allele frequencies were stable over the time period over which this study was conducted.

Pairwise F_{ST} values provided little evidence of genetic differentiation between sites or watersheds (Tables 16 and 17). There was no clear pattern of differentiation among watersheds. Hiilawe and Maili were significantly different, although the magnitude of differentiation was not

Hiilawe

Site	1	2	3	4	5	6
1						
2	0.002302					
3	0.001189	0.001114				
4	-0.002218	-0.001693	-0.003014			
5	0.001106	0.000578	0.000803	0.000065		
6	0.005809	0.003894	0.002926	-0.002091	0.002615	

Hanawi

Site	1	2	3	4	5	6
1						
2	0.000169					
3	0.000708	0.002363				
4	0.001323	0.00028	0.001399			
5	-0.005323	-0.003666	-0.002113	-0.003053		
6	-0.000355	-0.000673	-0.000242	0.001562	-0.005958	

Maili

Site	1	2	3	4	5	6
1						
2	0.005874					
3	-0.003494	0.000837				
4	0.011329	**0.012624**	**0.008944**			
5	0.009182	**0.007032**	0.003981	0.007385		
6	0.001494	0.002108	0.000771	**0.008838**	-0.000452	

Waimea

Site	1	2	3	4
1				
2	-0.0068			
3	-0.0088	-0.0035		
4	-0.0066	0.0031	-0.0038	

Table 16: Pairwise F_{ST} values for 4 watersheds on Hawaii and Oahu. Values in bold are significant after Bonferroni correction.

great (F_{ST} = 0.0005). Similarly, no clear longitudinal patterns of differentiation were found within watersheds. A few sites were significantly different from one another in pair-wise

comparisons within Maili watershed (Table 16), and a significant pattern of isolation by distance (IBD) was observed in the Hiilawe watershed ($r^2 = 0.1764$; Figure 58). No evidence of IBD was observed in Hanawi ($r^2 = 0.0013$) or Maili ($r^2 = 0.0003$), and significant autocorrelations among F_{ST} values were not detected at any distances (Figure 59).

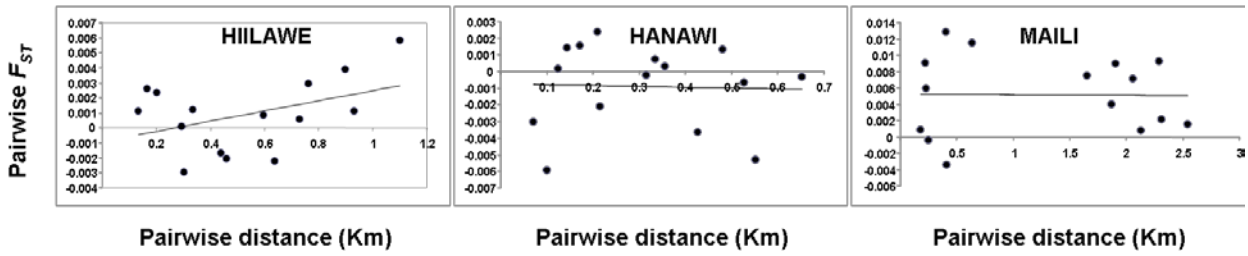

Figure 58: Pairwise F_{ST} estimates of genetic differentiation among sites according to pairwise distances (km) among sample sites in three watersheds on Hawaii. Statistical values are provided in the text.

Values from STRUCTURE recovered support for two distinct populations of *A. stamineus* among the sites sampled in the four study watersheds (Figure 59). However, both populations were present at each of the 22 sample sites. No support was found for spatial subdivisions within or between watersheds. No evidence was found for spatial subdivision within or between islands.

Elevation	High	Low
High		
Low	**0.001007**	

Position	Low	Mid	High
Low			
Mid	0.000264		
High	0.000807	0.000484	

Month	June	July	Aug	Sept	Oct	Nov
June						
July	**0.002037**					
Aug	0.001278	0.001119				
Sept	0.001126	**0.001318**	0.001348			
Oct	**0.015031**	**0.011432**	**0.013027**	**0.013835**		
Nov	**0.00223**	**0.002775**	0.002008	**0.002177**	**0.007808**	

Watershed	Waimea	Hiilawe	Hanawi	Maili
Waimea				
Hiilawe	-0.000494			
Hanawi	-0.000518	0.000489		
Maili	-0.000299	**0.000549**	-0.000018	

Table 17: Pairwise F_{ST} values by month and elevation and across 4 watersheds on Hawaii and Oahu. Values in bold are significant after Bonferroni correction.

***Relationships between genetic diversity and population densities*:** Positive correlations were consistently recovered between measures of *A. stamineus* genetic variation (NAA, A_r, Shannon diversity and *Neb*) and measures of *A. stamineus* demography (*A. stamineus* density, *Phi*, and *f*). The 22-site dataset identified positive correlations between *A. stamineus* density and NAA ($r = 0.805$, $p < 0.001$) and Shannon diversity ($r = 0.68$, $p < 0.001$) and a negative correlation with *Neb* ($r = -0.510$, $p < 0.001$). Positive correlations also were found between *A. stamineus* density and NAA ($r = 0.90$, $p < 0.001$), A_r ($r = 0.619$, $p < 0.001$), and Shannon diversity ($r = 0.791$, $p < 0.001$), and a negative correlation with *Neb* ($r = -0.763$, $p < 0.001$) in comparisons restricted to the 12 site, mark-recapture dataset. A positive correlation was also identified between *Phi* and NAA ($r = 0.799$, $p < 0.001$), A_r ($r = 0.600$, $p < 0.001$) and Shannon diversity ($r = 0.797$, $p < 0.001$), whereas a negative correlation was found between *Phi* and *Neb* ($r = -0.63$, $p < 0.001$). Conversely, a positive correlation was identified between *f* and *Neb* ($r = 0.619$, $p < 0.001$).

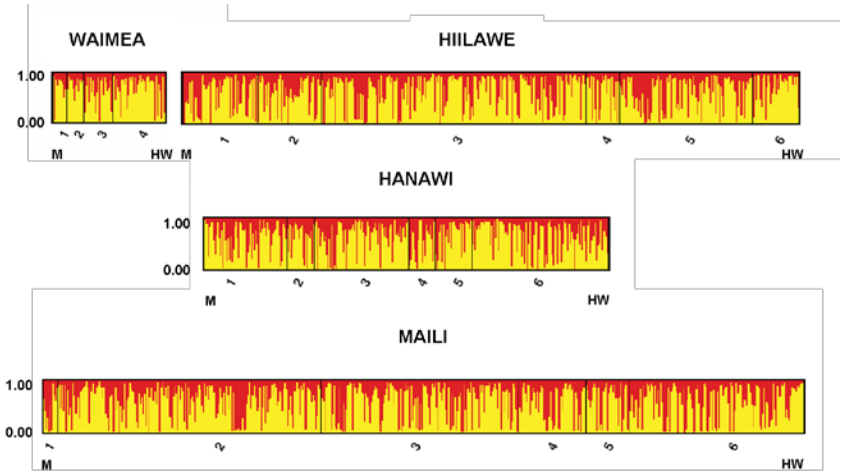

Figure 59: Bayesian estimates of genetic differentiation among sites within one watershed on Oahu (Waimea) and three watersheds on Hawaii (Hiilawe, Hanawi, Maili) with reference to longitudinal orientation to the mouth (M) and headwaters (HW), under conditions of *k* = 2; red = proportion of each individual's genotype assigned to population #1; yellow = proportion of each individual's genotype assigned to population #2); numbers on x-axes correspond to sample site locations in each watershed).

***Relationships between genetic diversity, goby population densities, and native species diversity*:** Informative but non-significant correlations were identified between measures of *A. stamineus* genetic diversity and total goby density ($0.463 < r < 0.51$; $p > 0.05$), as well as between *A. stamineus* genetic diversity and total goby richness ($-0.306 < r < -0.242$; $p > 0.05$) in the restricted analysis. A positive significant correlation was recovered between *A. stamineus* density and total goby density ($r = 0.531$, $p < 0.001$) in the 22-site analysis.

***Predictors of genetic diversity, population densities, and species diversity*:** Variables that were consistently identified as significantly correlated with diversity and density measures within and among watersheds were included in stepwise regression analyses to control for covariance (Tables 18 and 19). Stepwise regression models consistently identified distance to the stream mouth, water chemistry PC Factor 1 (WC_1), and *f* as predictors of diversity and densities for the mark-recapture subset of 12 sites (Tables 18 and 19). Distance to mouth was identified as a positive driver of A_r, and a negative driver of both total goby density and total goby richness. $WC_1$was a weak predictor of both Shannon diversity and total goby richness. Estimates of *f* were

identified as a strong predictor with negative influence on Shannon diversity as well as total goby density and *A. stamineus* density (Tables 18 and 19). In contrast, *f* was identified as a strong positive predictor of N*eb* (Table 18 and 19). Other variables were not consistently identified as predictors. Poeciliid density was identified as a strong predictor with positive influence on Shannon diversity, and NH_4 was selected as a positive predictor for *A. stamineus* density. *A. stamineus* density was selected as a positive predictor for NAA, and *K* was selected as a weak negative predictor of total goby richness. Overall model r^2 values ranged from 0 (Poeciliid density, allelic richness) to 0.9913 (Shannon diversity; Tables 18 and 19).

Table 18: Results of stepwise regression of *Awaous stamineus* genetic diversity for sites in 3 watersheds on Hawaii, against physiographic, abiotic environmental, and biotic factors. Shannon diversity = Shannon; average number of alleles per locus = Naa; Allelic richness = Ar; Effective population size = N*eb*; *A. stamineus* population density (VS) = visual survey; Top values reported in cells are partial correlation coefficients, with the direction of the relationship given as either positive (+) or negative (-). Full model r^2 values are also given in the second to bottom row. Bottom values reported in cells are p-values.

Hawaii only

	Naa	Ar	Shannon	N*eb*
Poeciliid density	0.1072 (+)		0.0871 (+)	0.1434 (-)
	0.028		0.0579	0.0921
Distance to mouth			0.2717 (+)	0.2929 (-)
			0.0048	0.0304
Water chemistry	0.0598 (+)			
	0.0644			
NH4				
A. stamineus density (VS)	0.4843 (+)		0.3551 (+)	
	0.0013		0.0091	
Full Model	0.7783	0	0.714	0.4363
	<0.0001	0	0.0004	0.0241

Stepwise regression models run with all 22 sites did not include *Phi*, *f*, *K*, or $L\infty$ as independent variables, as these values could only be estimated in the mark-recapture sites where multiple capture events were conducted. For these models, distance to the stream mouth was the most consistently identified predictor (Table 19). *A. stamineus* density was also consistently chosen as a predictor (Table 19). Distance to mouth was chosen as a positive predictor of Shannon diversity and Poeciliid density, and a negative predictor for N*eb*, total goby density, and total goby richness (Table 19). *A. stamineus* density was chosen as a positive predictor of NAA, Shannon diversity, and Poeciliid density, and as a negative predictor for N*eb* (Table 19). Poeciliid density was selected as a negative predictor for total goby richness, and water chemistry PC Factor 1 was only selected as a weak negative predictor for Poeciliid density (Table 19). Full model r^2 values ranged from 0.2083 (*A. stamineus* density) to 0.647 (NAA; Table 19).

Significant general linear models were recovered for A_r, Shannon diversity, total goby density, and Poeciliid density for the 12-site IMR subset and full 22-site dataset (Table 20). Significant models also were recovered for NAA, Shannon diversity, and N*eb* for the 12-site IMR dataset (Table 20). For the full dataset, distance to mouth was the stronger variable in the total goby density model, but watershed was the stronger variable in the Poeciliid density, A_r, and Shannon diversity models. In the IMR subset, watershed was the stronger variable in all significant models. The interaction term between distance to mouth and watershed was not significant in any model (Table 20).

All Sites

	Naa	Ar	Shannon	*Neb*	*A. stamineus* density (VS)	Poeciliid density	Goby density	Goby richness
Poeciliid density					0.2083 (+)			0.3887 (-)
					0.0431			0.0033
Distance to mouth			0.0715 (+)	0.2947 (-)		0.1615 (+)	0.3833 (-)	0.0777 (-)
			0.1285	0.006		0.0403	0.0033	0.1342
Water chemistry PC1						0.1111 (-)		
						0.1141		
NH4								
A. stamineus density (VS)	0.647 (+)		0.4523 (+)	0.2721 (-)	N/A	0.2083 (+)	N/A	N/A
	<0.0001		0.0012	0.0264		0.0431		
Full Model	0.647	0	0.5238	0.5668	0.2083	0.4809	0.3883	0.4664
	<0.0001	0	0.0018	0.0019	0.0431	0.0129	0.0033	0.0048

IMR Sites

	Naa	Ar	Shannon	*Neb*	*A. stamineus* density (IMR)	Poeciliid density	Goby density	Goby richness
Poeciliid density			0.2611 (+)					
			0.0171					
Distance to mouth		0.3832 (+)					0.4264 (-)	0.0073 (-)
		0.1017					0.0792	0.1422
Water chemistry PC1			0.0973 (+)					0.0531 (+)
			0.0026					0.1394
NH4					0.2530 (+)			
					0.1119			
A. stamineus	0.8901 (+)				N/A		N/A	N/A
	0.0004							
Phi								
f			0.6329 (-)	0.6185 (+)	0.4063 (-)		0.2762 (-)	
			0.0182	0.0206	0.0891		0.0837	
K								0
								0.0290 (-)
L∞								0.116
Full Model	0.8901	0.3832	0.9913	0.6185	0.6593	0	0.7026	0.9605
	0.0004	0.1017	0.0001	0.0206	0.0677	0	0.0482	0.0029

Table 19: Results of stepwise regression of *Awaous stamineus* genetic diversity and density, as well as native goby density and species richness for all 22 sites and IMR-only sites in 4 watersheds on Hawaii and Oahu, against physiographic, abiotic environmental, and biotic factors. Shannon diversity = Shannon; average number of alleles per locus = Naa; Allelic richness = Ar; Effective population size = N*eb*; *A. stamineus* population density (VS) = visual survey, (IMR) = individual mark-recapture; LULC = land use. Models are inclusive of *A. stamineus* density as an explanatory factor except for models of *A. stamineus* density. Regression models of Poeciliid density exclude Poeciliid density as an explanatory factor. Top values reported in cells are partial correlation coefficients, with the direction of the relationship given as either positive (+) or negative (-). Full model r^2 values are also given in the second to bottom row. Bottom values reported in cells are p-values.

Discussion: Attributing a shift in stream condition to military activity rather than other proximate factors requires use of assessment approaches that are capable of discriminating reach-scale (i.e., longitudinal) variation in stream condition. Naturally depauperate species diversity and longitudinal variation in assemblage-level characteristics complicate use of traditional ecological indicators for assessing and comparing the condition of sites within watersheds on oceanic islands. Genetic indicators are not constrained by low species diversity or longitudinal

variation in assemblage-level structure. Rather, genetic indicators may reflect stream condition relative to ocean-stream connectivity and dispersal geometry within and among watersheds (Blum et al. 2005, Waits et al. 2008, Lamphere and Blum 2012), where patterns of genetic variation are expected to bear signatures of population persistence and colonization relative to in-stream conditions (Waits et al. 2008) and watershed land use (Bagley et al. 2004, Blum et al. 2005, Blum et al. 2012). Thus patterns of genetic variation can reveal how environmental stressors affect individuals and populations (Schwartz et al. 2007) within spatially explicit frameworks (i.e., river-stream networks within watersheds).

This study was undertaken to examine within-watershed longitudinal patterns of genetic variation of a native goby relative to natural and anthropogenic conditions. This study was also conducted to determine the utility of genetic indicators for assessing and comparing the condition of sites within watersheds on oceanic islands. To meet our objectives, we characterized longitudinal patterns of (1) genetic diversity and effective population size; (2) mark-recapture estimates of population size, condition and individual movement; (3) snorkel survey estimates of population densities and assemblage-structure; and (4) site-specific water chemistry and habitat conditions. We also examined relationships between in-stream, watershed-scale, and island-wide conditions.

Our findings indicate that demographic and genetic variation of *A. stamineus* correspond to site-specific and watershed-scale conditions. Some of the observed variation is attributable to longitudinal position within the watershed, where a combination of results suggests that inland populations are more susceptible to extirpation than populations located closer to sources of colonization. However, our results also indicate that site-specific environmental conditions may supersede the importance of longitudinal distance in structuring within-watershed patterns of genetic diversity. Additionally, our results suggest that patterns of genetic diversity may reflect indirect effects arising from conditions elsewhere in the watershed. As migratory species, native stream fauna travel through lower watershed regions to reach habitat suitable for subsequent maturation and spawning. Consequently, the condition of resident populations may be attributable to the immediate environment and prior exposure to stressors experienced during upstream immigration. For example, predation of immigrating postlarvae by Poeciliids in lower watershed reaches could have lasting effects on population densities and genetic diversity elsewhere in a watershed. Non-native Poeciliid fish also may influence resource availability through competition and trophic cascades (Holitzki et al. 2013). Thus the observed responses to water chemistry and Poeciliid presence are most likely attributable to the combined influence of immediate environmental conditions and residual (yet lasting) effects of prior exposure that result in watershed-scale signatures of response.

We hypothesized that populations of *A. stamineus* located further inland and at higher elevations are demographic sinks because the likelihood of persistence might decrease with distance from sources of immigrating postlarvae. Population persistence and genetic structure of benthic stream fish can be significantly affected by longitudinal position and altered connectivity (Fagan 2002, Hänfling and Weetman 2006, Waits et al. 2008, Lamphere and Blum 2012). Source-sink dynamics, for example, can result in upstream populations representing a genetic subset of downstream populations (Hänfling and Weetman 2006, Waits et al. 2008, Lamphere and Blum 2012). Thus, declining genetic diversity with longitudinal distance can be indicative of

All sites

	Independent variables	Sum of squares	Mean square	F-value	p-value	r2
***A. stamineus* density (VS)**	Model	0.246	0.062	1.31	0.308	0.247
	Distance to mouth	0.039	0.039	0.83	0.376	
	Watershed	0.192	0.064	1.36	0.290	
Goby density	Model	2.371	0.593	3.28	**0.038**	0.450
	Distance to mouth	1.026	1.026	5.67	0.030	
	Watershed	0.176	0.059	0.32	0.808	
Goby richness	Model	7.643	1.911	2.77	0.063	0.409
	Distance to mouth	3.810	3.810	5.53	0.032	
	Watershed	6.412	2.137	3.1	0.056	
Poeciliid density	Model	7.766	1.942	7.85	**0.001**	0.649
	Distance to mouth	1.038	1.038	4.2	0.056	
	Watershed	7.286	2.429	9.82	0.001	
Naa	Model	74.328	18.582	1.66	0.206	0.281
	Distance to mouth	2.922	2.922	0.26	0.616	
	Watershed	73.876	24.625	2.2	0.126	
Ar	Model	0.650	0.163	6.36	**0.003**	0.600
	Distance to mouth	0.021	0.021	0.83	0.375	
	Watershed	0.586	0.195	7.64	0.002	
Shannon	Model	0.133	0.033	4	**0.018**	0.485
	Distance to mouth	0.017	0.017	1.99	0.176	
	Watershed	0.132	0.044	5.32	0.009	
Neb	Model	4013.109	1003.277	2.29	0.107	0.379
	Distance to mouth	926.343	926.343	2.12	0.166	
	Watershed	1828.147	609.382	1.39	0.284	
Skew	Model	5.891	1.473	1.08	0.408	0.249
	Distance to mouth	0.017	0.017	0.01	0.913	
	Watershed	4.057	1.352	0.99	0.429	
Kurtosis	Model	5.178	1.294	0.18	0.945	0.066
	Distance to mouth	4.611	4.611	0.63	0.445	
	Watershed	3.259	1.086	0.15	0.928	

IMR sites

	Independent variables	Sum of squares	Mean square	F-value	p-value	r2
***A. stamineus* density (IMR)**	Model	0.223	0.056	4.1	0.051	0.701
	Distance to mouth	0.000	0.000	0.01	0.913	
	Watershed	0.206	0.069	5.06	0.036	
Goby density	Model	2.000	0.500	2.87	0.106	0.622
	Distance to mouth	0.269	0.269	1.55	0.254	
	Watershed	0.351	0.117	0.67	0.596	
Goby richness	Model	8.488	2.122	6.11	**0.019**	0.777
	Distance to mouth	1.571	1.571	4.53	0.071	
	Watershed	7.536	2.512	7.24	0.015	
Poeciliid density	Model	7.628	1.907	13.18	**0.002**	0.883
	Distance to mouth	1.325	1.325	9.15	0.019	
	Watershed	7.071	2.357	16.28	0.002	
Naa	Model	140.730	35.182	12.11	**0.003**	0.874
	Distance to mouth	2.172	2.172	0.75	0.416	
	Watershed	129.533	43.178	14.86	0.002	
Ar	Model	0.492	0.123	15.82	**0.001**	0.900
	Distance to mouth	0.015	0.015	1.97	0.203	
	Watershed	0.469	0.156	20.13	0.001	
Shannon	Model	0.140	0.035	32.16	**0.000**	0.948
	Distance to mouth	0.009	0.009	8.03	0.025	
	Watershed	0.133	0.044	40.81	<.0001	
Neb	Model	1227.558	306.890	12.97	**0.002**	0.881
	Distance to mouth	54.856	54.856	2.32	0.172	
	Watershed	1223.769	407.923	17.23	0.001	
Skew	Model	7.044	1.761	1.87	0.220	0.517
	Distance to mouth	0.807	0.807	0.86	0.385	
	Watershed	3.925	1.308	1.39	0.323	
Kurtosis	Model	15.809	3.952	1.05	0.457	0.411
	Distance to mouth	0.345	0.345	0.09	0.773	
	Watershed	14.906	4.969	1.31	0.354	
Phi	Model	0.151	0.038	3.91	0.056	0.691
	Distance to mouth	0.035	0.035	3.64	0.098	
	Watershed	0.146	0.049	5.04	0.036	
f	Model	0.068	0.017	2.85	0.108	0.619
	Distance to mouth	0.032	0.032	5.28	0.055	
	Watershed	0.032	0.011	1.79	0.237	
K	Model	0.003	0.001	4.73	0.084	0.780
	Distance to mouth	0.002	0.002	7.5	0.052	
	Watershed	0.003	0.001	5.32	0.075	
***L*∞**	Model	27815.743	9271.914	0.75	0.578	0.359
	Distance to mouth	2124.662	2124.662	0.17	0.700	
	Watershed	17936.208	8968.104	0.72	0.539	

Table 20: General linear model results for *Awaous stamineus* genetic diversity and demography, as well as total goby density, goby richness, and Poeciliid density.

directionally-biased dispersal, where upstream reaches are receiving immigrants from lower reaches (Slatkin 1993, Slatkin 1995, Hänfling and Weetman 2006, Lamphere and Blum 2012). Distance to the stream mouth was consistently recovered as a positive predictor in stepwise regression models of *A. stamineus* genetic diversity, which provides support for the premise that occupancy of upper reaches is being sustained by a sustained influx of immigrants via directionally-biased dispersal. However, low levels of genetic differentiation indicate that *A. stamineus* populations are not highly fragmented or isolated within watersheds, which in turn suggests that the likelihood of persistence is not solely a function of longitudinal position. This inference is supported by *A. stamineus* population census data, which indicates that size structure does not differ according to longitudinal position.

Longitudinal patterns of genetic diversity under conditions of high connectivity and continuous size structure suggests that extensive directional movement is a unifying force within watersheds. Mark-recapture estimates indicate, though, that juvenile and adult *A. stamineus* do not move extensively within watersheds. Demographic and genetic studies of other benthic stream fish with low juvenile and adult movement potential have recovered highly structured patterns of genetic diversity and differentiation (Hänfling and Weetman 2006, Lamphere and Blum 2012). Species of sculpin (genus *Cottus*), which have low levels of inter-population movement across distances as small as 0.5 km, exhibit highly structured populations within watersheds (Hänfling and Weetman 2006, Lamphere and Blum 2012). Genetic diversity in sculpin is also highly correlated with longitudinal position across watershed and reach-level spatial scales (Hänfling and Weetman 2006, Lamphere and Blum 2012). The patterns observed in *A. stamineus* are more like those found in some freshwater mussels with sessile mature life stages and highly dispersive early life stages (Ferguson et al. 2013). If juvenile and adult *A. stamineus* do not move among sites, then population connectivity within watersheds is likely sustained by directional movement of postlarvae, where propagule pressure does not necessarily diminish with longitudinal position. The importance of longitudinal position likely differs among watersheds, and possibly among islands, if the nature of in-stream physical barriers reflects island age in archipelagos (i.e., where geomorphic changes are driven by erosion). The strength of biotic barriers also likely varies among watersheds and islands, where the density and distribution of predators can be contingent on habitat availability (i.e., the extent of predatory *Eleotris sandwicensis* in estuarine reaches) and historical legacies of human interventions (i.e., the introduction of predatory non-native species).

Weak genetic differentiation and extensive movement within watersheds does not necessarily preclude the possibility that upstream populations are demographic sinks. Even if mortality in upstream populations is greater than levels of self-recruitment, population size and genetic diversity can be buoyed by immigration (Keller et al. 2001, McMillan et al. 2006, Waits et al. 2008, Hogan et al. 2012). Pearson correlations indicate that apparent recruitment declines with distance to the stream mouth. Stepwise regression models of all sites also indicate that effective population size declines and that Shannon diversity increases with distance to the stream mouth (Tables 18 and 19). Together, these relationships suggest that population stability declines with distance from the stream mouth, but that instability is likely tempered by the influx of postlarvae. Evidence of temporally stable patterns of within-watershed demographic and genetic variation support this inference.

We also hypothesized that populations of *A. stamineus* located further inland and at higher elevations could be a genetic subset of coastal and lower elevation populations due to adaptive differences in climbing ability and predator avoidance (i.e., the selective sieve hypothesis). Frequency-based analyses of populations of *A. stamineus* did not recover evidence of hierarchical genetic structure within watersheds, though a Bayesian cluster analysis estimated that two subpopulations of *A. stamineus* exist in the study watersheds. However, individuals from both subpopulations were consistently present across all sites, suggesting the possibility of co-occurring evolutionary lineages (i.e., that might represent distinct life history variants, as described above). Evidence of genetic differentiation co-varying with geographic distance was recovered for one watershed over a distance of only ~1.2km, which is more suggestive of isolation-by-distance or other factors that might result in dispersal limitation (e.g., in-stream barriers) rather than selection influencing the longitudinal distribution of phenotypes. Thus, at least among the select watersheds studied here, multilocus microsatellite variation in *A. stamineus* did not provide evidence supporting the proposed selective sieve hypothesis.

We did find evidence that variation among populations of *A. stamineus* corresponded to site-specific conditions, where genetic diversity varied in response to environmental impairment (Hughes and Noss 1992, Blum et al. 2012). The primary indicators of environmental impairment used in this study were water chemistry parameters related to nutrient conditions and the presence of invasive Poeciliid fish. WC_1, which is a composite measure of NO_3 and SRP conditions, was consistently chosen as a predictor of genetic diversity and effective population size by stepwise regression models, with positive and negative directions of influence, respectively (Tables 18 and 19). Poeciliid density was also consistently chosen by stepwise regression models, and consistently had a positive influence on genetic diversity and a negative influence on effective population size (Tables 18 and 19). A positive relationship between Poeciliids and genetic diversity in fine-scale longitudinal comparisons seemingly contradicts the relationships observed in archipelago-wide comparisons among watersheds, which suggest that the presence of Poeciliids acts to reduce genetic diversity (see section 5.1.2). Comparisons to measures of residency and immigration, such as effective population size, help reveal the basis of unexpected and ostensibly contrary relationships with impairment (Waits et al. 2008).The negative relationship with effective population size suggests a more nuanced relationship between Poeciliids and genetic diversity of native fish, where elevated genetic diversity across longitudinal transects may be a consequence of Poeciliids reducing resident population size and increasing the proportion of immigrants at (or traveling through) particular sites. A similar dynamic might arise as a consequence of impaired water quality conditions. It is also possible that the observed relationships with water conditions and Poeciliids reflect watershed-scale signatures of response arising from indirect effects or prior exposure. Evidence that apparent recruitment is significantly higher in Hanawi watershed, where Poeciliids are absent (Figure 57), supports the inference that population persistence is less likely in watersheds that harbor Poeciliid fish. Results from GLM analysis also indicate that variation in effective population size, apparent recruitment, and Shannon diversity is attributable to differences among watersheds (Table 20).

Though this study indicates that demographic and genetic variation in *A. stamineus* vary more among watersheds than within watersheds, it nonetheless shows that *A. stamineus* respond to within-watershed differences in natural and anthropogenic conditions. These findings serve to

reiterate the value of genetic indicators as tools for understanding and assessing spatially-explicit responses to site-specific environmental conditions. By providing information on both demographic and genetic variation (Leidner and Haddad 2011, Lamphere and Blum 2012), this study also revealed that outcomes of natural and anthropogenic conditions are likely mitigated by the influx of postlarvae, which suggests that the recovery of extirpated populations at local sites or whole watersheds is contingent on both improvement of in-stream conditions (i.e., control or removal of Poeciliids) and adequate propagule pressure for recolonization by postlarvae (i.e., restoration of ocean-stream connectivity).

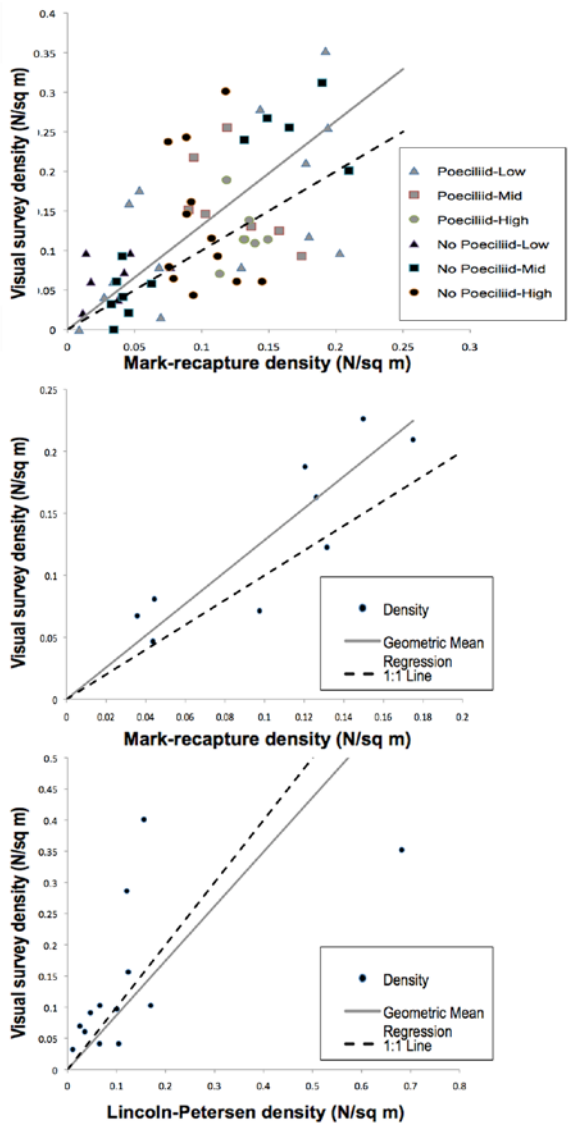

Figure 60: Geometric mean regression of IMR and visual surveys (individual sampling events) with 1:1 line for reference (top); Geometric mean regression of IMR and visual surveys averaged per site with 1:1 line for reference (middle); Geometric mean regression of Lincoln-Peterson batch mark-recapture and visual surveys with 1:1 line for reference (bottom).

Comparison of conditions in watersheds on Hawaii to Waimea watershed and other watersheds on Oahu (see section 5.1.2) indicates that favorable in-stream conditions are not necessarily sufficient for sustaining local populations of native fish. Depressed goby densities in remote, forested watersheds on Oahu suggest that propagule pressure is weak across the island. Evidence from otolith microchemistry (see section 5.1.2) and coupled biophysical modeling (see section 5.0.5) also suggests that contributions from other islands to the propagule pool around Oahu are likely small. With the majority of the propagule pool drawing from proximate sources, where most larvae remain in freshwater or nearshore environments like estuaries or freshwater plumes, low rates of recovery will likely occur following improvement of local in-stream conditions (i.e., rates of recovery are likely contingent on propagule pressure). Recovery of impaired populations on Oahu could be accelerated through a cross-watershed (or cross-island) restoration program intended to systematically improve adult reproduction, recruitment and survivorship. Recovery might also be promoted through cross-island translocations. Further studies, perhaps involving experimental manipulations of in-stream conditions and translocations, will be necessary to identify the sequence and scale of interventions necessary for restoring populations of at-risk species in Pacific island streams.

5.1.4 Mark-recapture Calibration of Snorkel Surveys

***Comparison of population density estimates*:** Densities of *A. stamineus* were considerably lower in Waimea watershed than in the three watersheds on Hawaii (all, $p < 0.001$; Tables 4 and 21). A total of only 92 individual fish were marked across the three IMR sites in Waimea watershed, with only 20 total recaptures (Table 4). Of these, 56 marked fish, and 10 recaptures were at the longitudinally highest site (Table 4). This yielded IMR population density estimates of 0.017 +/- 0.009 for the low site, 0.019 +/- 0.009 for the mid site, and 0.028 +/- 0.02 for the high site (Table 21). The number of recaptures was also small for the batch mark-recapture (BMR) study done at the high site, with only one recapture out of 9 marked fish (Tables 4 and 21). Consequently, all comparisons between visual survey estimates and mark-recapture estimates were limited to sites in Hiilawe, Hanawi, and Maili watersheds (Table 21).

Site	Island	2010 VS	IMR	2011 VS	BMR
Hiilawe-Low	Hawaii	0.204	0.108		
Hiilawe-Mid	Hawaii	0.684	0.412		
Hiilawe-High	Hawaii	0.178	0.251	0.174	0.060
Hanawi-Low	Hawaii	0.168	0.086		
Hanawi-Mid	Hawaii	0.113	0.106	0.250	0.085
Hanawi-High	Hawaii	0.540	0.320		
Maili-Low	Hawaii	0.620	0.496	0.300	0.260
Maili-Mid	Hawaii	0.455	0.337		
Maili-High	Hawaii	0.326	0.354		
Waipio-High	Hawaii			0.267	0.478
Hakalau-Low	Hawaii			0.100	0.162
Alelele-Low	Maui			1.517	0.432
Waihee-Low	Maui			0.267	0.164
Honokohau-Low	Maui			0.100	0.271
Halawa-Mid	Molokai			0.933	0.321
Honouli Wai-High	Molokai			0.433	0.330
Keaahala-Mid	Oahu			1.250	3.806
Kahana-Mid	Oahu			0.233	0.113
Waimea-Low	Oahu	0.046	0.016		
Waimea-Mid	Oahu	0.050	0.019		
Waimea-High	Oahu	0.125	0.028	0.077	0.024

Table 21: Population density estimates from visual surveys conducted in 2010 (2010 VS), individual mark-recapture (IMR), visual surveys conducted in 2011 (2011 VS), and batch mark-recapture (BMR) for watersheds across the Hawaiian Islands.

Density estimates from IMR and visual surveys were significantly correlated whether the analysis involved averaging data for each site across samples ($N= 9$, $r^2 = 0.773$) or involved regressing each sample ($N= 60$, $r^2 = 0.405$; Table 22). The equations for GM regressions of site-averaged and per-sample data were similar, with slopes of 1.284 +/- 0.227 and 1.317 +/- 0.151, respectively (Table 22). The density estimate from visual surveys exceeded that from IMR for most samples and sites (Figure 60), resulting in slope estimates significantly higher than 1 for both the site-averaged data (H_0: slope = 1.0, $\alpha = 0.05$, $p = 0.021$) and the per-sample data ($p < 0.001$; Table 22).

Method	r^2	Slope	SE	CI Low	CI High	p-value
Lincoln-Peterson	0.415	0.873	0.151	0.540	1.206	0.419
Individual mark-recapture	0.405	1.317	0.075	1.167	1.468	<0.0001
Individual mark-recapture, site avg	0.773	1.284	0.096	1.057	1.511	0.021

Table 22: Regression results for density estimates from visual survey versus mark-recapture. Regressions were performed on log (x+1) transformed densities. The slope is calculated without an intercept. Standard error (SE) is approximated as the standard error of the ordinary least regressions model. Significant p-values ($\alpha < 0.05$) indicate that the slope is different from 1. Lincoln-Peterson corresponds to the batch mark-recapture (BMR) method.

Population density estimates from visual surveys were also significantly correlated to those from Lincoln-Peterson BMR (N= 13, $r^2 = 0.415$; Table 22). Visual survey estimates were higher than Lincoln-Peterson BMR estimates at 8 of 13 sites (Figure 60), yielding a GM regression slope of 0.873 +/- 0.333 that was not different from 1 ($p = 0.419$; Table 22).

The general linear model of the residuals from the GM regression of visual surveys and IMR indicated significant differences among capture events ($p = 0.044$; Table 23). Residuals for samples indicated that density estimates from visual surveys were greater than estimates from IMR in Hanawi (mean = 0.0122), however densities estimates from IMR were greater than estimates from visual surveys in both Hiilawe and Maili (means = -0.003 and -0.291, respectively). The differences in ratios among the watersheds were not significant ($p > 0.05$; Table 23), although residuals for capture events from October and November were significantly ($p < 0.05$) more positive (visual > IMR; 0.034 and 0.031, respectively) than the residuals from June (-0.059; Table 23).

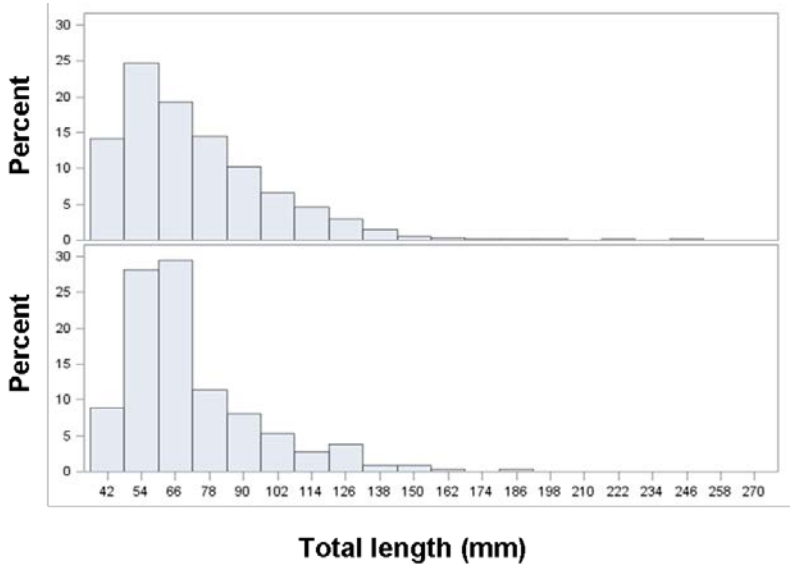

Figure 61: Histogram of total length of observed fish from visual surveys (bottom) and IMR (top) shown as percent of total observed population from individual size bins.

Across all sites, the reported size distribution of fish recorded in the visual surveys differed from those collected for mark-recapture ($p = 0.004$), with visual samples under-reporting larger (>150 mm) individuals relative to mark-recapture (Table 24). As a result, the distribution of sizes in the

mark-recapture dataset is more skewed (skew = 1.705), with a longer right tail, than the visual survey dataset (skew = 1.471; Figure 61). The difference in skew between the two sampling methods was consistent across the covariates tested, including watershed, Poeciliid presence, and capture event (Table 24).

Discussion: Density estimates from visual surveys were significantly correlated with estimates from individual mark-recapture, which represents an effort-intensive "in hand" method that produces high-quality demographic data (Table 22). Because visual surveys overestimated density and under-sampled large size classes relative to IMR (Figure 62) some calibration would be necessary to compare absolute densities or size distributions across these methods. Our results indicate that IMR-based adult density estimates can be approximated by dividing a single VS estimate by 1.317, or the average of several VS estimates from the same site by 1.284. Because the degree of difference between visual surveys and IMR was significantly correlated with capture event (Table 23), time-specific correction factors likely would be most effective for comparing absolute values. Estimates from batch mark-recapture were not significantly different from visual survey estimates, though greater variation was found across sites (Figure 60). Despite the need for calibration, visual surveys are a more efficient approach to estimate population density and size structure than other rapid survey methods, such as batch mark-recapture, for *A. stamineus* and possibly for other benthic stream fishes as well.

To our knowledge, this study represents the first direct comparison of visual surveys and IMR. Comparisons of visual surveys to another "in hand" method (e.g., depletion electrofishing) supports our result that visual surveys correlate well with other survey methods under a variety of conditions (Mullner et al. 1998, Wildman and Neumann 2003). However, in contrast to our findings, comparisons to depletion electrofishing found that visual surveys underestimated fish

Source	DF	Sum of squares	MSE	F-Value	p-value
Poeciliid presence	1	0.005	0.005	1.41	0.242
Watershed	2	0.018	0.009	2.29	0.113
Position	2	0.009	0.005	1.18	0.318
Capture	6	0.055	0.009	2.39	0.044
Site	3	0.023	0.008	2.01	0.126
Overall model	14	0.110	0.008	2.05	0.035
Error	45	0.173	0.004		
Corrected total	59	0.283			

Table 23: General linear models (GLM) of y-axis residuals from the GLM regressions (r^2 = 0.389) of visual survey and individual mark-recapture data.

densities. This discrepancy is likely attributable to prior studies relying on visual estimates from simple counts, rather than the sub-sampling approach of the point-quadrat method that we used. Where the point-quadrat method may over- or under-estimate true densities, the total count method can only result in an undercount unless observers count the same individual two or more times.

Comparisons across the four sites where individual mark-recapture, batch-mark recapture, and visual surveys were conducted indicate that outcomes of all three methods are correlated,

although BMR tends to underestimate densities and VS most closely matches IMR when values are averaged over multiple surveys (Figure 60). Though BMR generally overestimates population sizes (Chapman 1951), we found that the approach underestimated densities relative to IMR at all four sites (Figure 60). We also found that density estimates averaged across surveys were very similar to IMR estimates (Figure 62) in three of the four study watersheds. Our findings in Waimea watershed are the exception. This is likely a result of fewer sampling events having been conducted at Waimea, which resulted in estimates having greater variance than density estimates for sites in the other three watersheds. Sites in Waimea also were more turbid than sites in other watersheds, which possibly contributed to lower VS density estimates as well as lower capture and recapture success.

Method	N	Mean	SD	Min	Max	Skew	Kurtosis	KS	Ksa	D	p-value
Individual mark-recapture	2236	73	27	41	274	1.705	5.479				
Visual survey	394	70	23	42	180	1.471	2.360	0.034	1.751	0.096	0.004

Table 24: Statistical comparison of the observed size distributions from visual surveys and individual mark-recapture. A significant p-value ($\alpha < 0.05$) in the Kolmogorov-Smirnov test (KS) indicates a difference in the distribution of size structure between the two sampling methods.

Comparisons also indicate that the size of fish, habitat type, and capture event can mediate the correlation between estimates from visual surveys and mark-recapture. Visual surveys yielded higher density estimates than IMR overall (Figure 60) and for most size classes (TL = 40-150 mm) but lower estimates for large fish (> 150 mm; Figure 60). Together, these results suggest that large fish were rare but had greater capture probability. Prior studies, however, have found that visual survey estimates were more efficient with larger fish (Wildman and Neumann 2003, Thurow et al. 2006). Though we found no evidence of significant observer bias in our dataset, prior studies have reported a positive correlation between observer experience and the numbers of individuals and species detected (Williams et al. 2006).

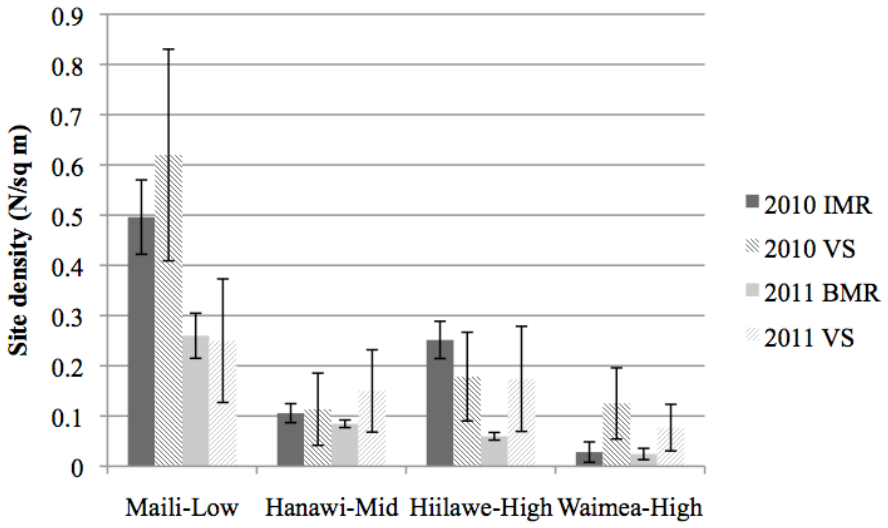

Figure 62: Density estimates and standard errors (error bars) for batch mark-recapture (BMR), individual mark-recapture (IMR) and visual surveys for four study watersheds. BMR are Lincoln-Peterson estimates using Chapman corrections for overestimates. IMR values are average of POPAN N-hat values for each sample event. 2010 visual surveys are the average and standard error of surveys collected during each IMR capture event. 2011 surveys are single samples, with the standard error reflecting variation among quadrats surveyed.

The differences in the residual densities by watershed and capture event indicate that any correction factors applied to estimates from visual surveys should be time-specific and perhaps site-specific. Visual survey results can be influenced by habitat characteristics, including stream size, mean current velocity, cobble substrate type, and water turbidity, and by the percentage of habitat sampled (Peterson and Rabeni 2001). Visual surveys also typically exhibit greater heterogeneity in detection probability across sites than "in hand" methods (Williams et al. 2006). Calibration using an independent method of estimating population size (i.e., a "dual gear" approach), can correct for these deficiencies (Hankin and Reeves 1988). Use of IMR on a subset of sites can thus produce calibrated population estimates that are superior to those derived from visual surveys alone (Sutherland 2006, Carrier et al. 2009), with little additional time investment.

This study addressed differences in density and size structure between point-quadrat visual surveys and mark recapture using hand-nets. Future work should expand efforts to compare the accuracy and biases of other common methods, including transect-based visual samples (Young and Young 1998, Kido 2002) and depletion sampling. Additionally, efforts to quantify the minimum number of quadrats necessary to produce accurate estimates of density, as well as presence/absence, should be pursued. Doing so would provide guidance for biologists aiming to select the sampling and estimation methods that are most appropriate for their specific research questions or management concerns.

Overall, our results indicate that visual surveys can offer a reliable index of relative abundance with less effort than either batch or individual mark-recapture approaches. Though IMR is widely viewed as the more reliable and informative means to assess population structure, especially if demographic data are of interest, it requires a significantly greater investment of time and expertise. The capacity to quickly generate reliable data makes visual surveys a low-cost, easy-to-implement option for a variety of studies, particularly for those covering large geographic areas or employing citizen scientist and volunteer monitoring groups. However, dual-gear calibration could be used to generate estimates of absolute abundance, if desired, while minimizing the need for effort-intensive IMR sampling.

6 Conclusions and Implications for Future Research and Implementation

Department of Defense resource managers are in need of improved stream assessment protocols to support efforts intended to mitigate activities that threaten watersheds on Pacific islands. Here we have shown how mobilizing knowledge of insular freshwater streams, including interactions with near-shore marine environments, can improve understanding and assessment of responses to natural and anthropogenic stressors (Jenkins et al. 2010). We have also shown how diagnosing conditions according to criteria that reflect the defining physical, biological and cultural dimensions of oceanic island streams can help DoD managers promote the recovery and integrity of at-risk native species.

The overall objective of this project was to develop and demonstrate genetic approaches for assessing the condition of Pacific island streams. Traditional assemblage-based protocols emphasize measures that do not capture the cultural importance of native species (e.g., total species richness), and assessment protocols that rely on measures of native assemblages are likely to underestimate impairment because the biota of Pacific island streams is naturally depauperate and tightly linked to oceanic environments. We proposed that measures of genetic variation can overcome these limitations by revealing the influence of environmental stressors on individuals and populations according to processes that sustain native species in degraded waterways, including interactions with near-shore marine environments. Evaluating the utility of genetic methods as tools for assessing stream condition required more thorough knowledge of the demographic and ecological processes- especially dispersal and sensitivity to environmental stressors- that give rise to patterns of genetic variation within species that serve as biotic indicators. Focusing on the Hawaiian Islands, we undertook complementary studies of historical biogeography and population connectivity of native amphidromous fishes to identify the most appropriate spatial scale for managing at-risk species and stream ecosystems. We also assessed hierarchical patterns of genetic variation in relation to local and watershed-scale environmental conditions to determine the degree to which measures of genetic diversity, population density, and assemblage structure reflect in-stream habitat degradation and watershed land use patterns.

Meeting our target objectives has resulted in the advancement of basic knowledge of oceanic island stream ecosystems and an unprecedented assessment of stream conditions across the Hawaiian archipelago. Several important conclusions can be drawn from our findings:

Integrative protocols tailored to capture estimates of genetic variation alongside measures of population density and species diversity can provide a robust basis for management and restoration of streams and conservation of constituent biota in Hawaii and elsewhere. Information on genetic variation provides distinct and valuable perspectives on responses to impairment and processes that sustain populations in degraded waterways. Because information on genetic variation can complement information on population densities and assemblage structure, integrative assessment approaches can be more effective than traditional assessment approaches.

Site-specific and watershed-scale conditions may supersede the importance of physiographic conditions in structuring genetic, demographic and assemblage variation of native

amphidromous species in oceanic island streams. Physiographic features that control carrying capacity (e.g., watershed area) and habitat availability (e.g., longitudinal position) are primary determinants of genetic diversity, population densities and native species diversity. Invasive species and land use intensification, however, diminish densities and diversity of native species, where the condition of local populations is attributable to the immediate environment and stressors elsewhere in the watershed that result in watershed-scale signatures of response.

The influx of postlarvae may sustain local populations in degraded waterways, thus the recovery of extirpated populations at local sites or whole watersheds is contingent on both improvement of in-stream conditions (i.e., control or removal of Poeciliids) to promote maturation and reproduction of residents and adequate propagule pressure for recolonization by postlarvae (i.e., restoration of ocean-stream connectivity).

Immigrant pools can potentially draw from populations across the archipelago, but among-island dispersal has little influence on local population dynamics. Though even a modest amount of admixture in immigrant pools can result in signatures of weak genetic differentiation and high gene flow, there are telltale signs that among-island propagule pressure is not high enough to rescue local populations from watershed or island-wide degradation. Thus, at-risk species are likely more susceptible to local environmental impairment than is now thought.

Aggregate effects can arise from local and watershed-scale degradation, where the cumulative influence of biotic or abiotic stressors can disrupt processes that promote the persistence of native fishes across entire islands. Categorical comparisons of watersheds across the archipelago showed that forested watersheds harbor higher densities of native fish populations, except on Oahu. Though predominantly forested watersheds generally support greater native species richness and more genetically diverse populations of native stream fishes, population densities of all native stream fishes were depressed across Oahu. This suggests that impairment not only diminishes local population size, but also reduces immigrant pools that are predominantly composed of larvae originating from local watersheds (i.e., on the same island). Weak genetic differentiation suggests extensive exchange occurs among islands, but immigration from other islands is not sufficient to compensate for the reduced productivity of impaired populations on Oahu. Should impairment persistent, negative feedback cycles driven by depressed productivity and immigration could end in extirpation of at-risk species across the entire island.

The condition of watersheds under military stewardship is comparable to areas elsewhere on Oahu. Unlike prior assessments, the design of this study enables comparisons to be drawn within and among watersheds on Oahu as well as among watersheds across islands. Areas under military stewardship harbor population densities and levels of diversity comparable to other watersheds on Oahu, with patterns of variation suggesting that the condition of areas under military stewardship largely reflect underlying land use and the presence of invasive species.

Meeting our target objectives has also resulted in novel perspectives on management of oceanic island stream ecosystems to sustain at-risk native species. With observed rates of local recruitment (i.e., through facultative amphidromy and retention of marine larvae) comparable to some of the highest estimates of self-recruitment for marine species with dispersive larvae,

stewardship and management of oceanic island stream ecosystems should not rely on among-island immigration as a tool or strategy for recovery of at-risk populations. Recommendations to temper reliance on among-island dispersal for recovery of at-risk populations is a departure from the prevailing notion that local populations of native freshwater fishes can be rescued by immigration from populations elsewhere in the Hawaiian archipelago (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998). This hypothesis, which largely originated from evidence of weak genetic differentiation and high gene flow among islands (Fitzsimons et al. 1990, Zink et al. 1996, Chubb et al. 1998, Bebler and Foltz 2004) does not account for estimates of immigration relative to local population sizes, nor does it account for evidence of extirpation and persistently depressed populations in the archipelago (Timbol and Maciolek 1978, Luton et al. 2005). A modest amount of immigration can result in signatures of weak genetic differentiation and high gene flow, but nonetheless have little influence on local population dynamics (Hodges and Allendorf 1998). Greater success will likely come from approaches that promote population recovery by improving propagule pressure originating from local or proximate sources. This could be done by controlling invasive species or improving the quality and availability of habitat to enhance productivity within and among watersheds in a region. Addressing conditions on Oahu, where at-risk species are rare to absent, will likely require more inclusive approaches that promote recovery across the island and resources on other islands (e.g., Molokai) that could support translocation programs.

Implementing integrative assessment protocols would provide highly informative performance measures to track outcomes of watershed management in the Hawaiian Islands and elsewhere in the Pacific. Based on our findings, we recommend that managers implement an integrative protocol that includes (1) snorkel survey approaches for collecting data on population, assemblage and community structure; and (2) genetic approaches for collecting multi-locus microsatellite data on diversity and differentiation in two or more ecologically contrasting sentinel species. We also recommend that managers obtain data on in-stream abiotic conditions. If possible, managers should examine traditional and stable isotope measures of nutrient enrichment, though some preference should be given to stable isotope measures because of the added benefits of utilizing the data for examining trophic responses to degradation. Few barriers exist to putting integrative protocols in to action. The accessibility of new tools effectively addresses critical gaps in resource availability that would otherwise limit adoption and application of integrative assessment protocols. For example, species-specific panels of nuclear microsatellite markers have been developed for two native species that serve as biotic indicators. Personnel also could be readily trained in field methods and data analysis. For example, obtaining materials for genetic analysis represents a simple extension of snorkel survey protocols that are already widely used in Hawaii. As with traditional assessment approaches that require assemblage-level data on benthic macroinvertebrates, specialized facilities would be engaged to conduct lab-based work (i.e., to eliminate the need for outfitting a specialized facility to generate genetic data, which can cost upwards of $500,000 with comparable annual maintenance costs for salaries and supplies). Generating genetic data would be an additional expense beyond the costs of current protocols that emphasize field-based data collection on population densities through snorkel surveys. However, the additional expense would be minor (i.e., a 10-20% increase) considering that the majority of costs involved with environmental assessments of oceanic island watersheds are related to field operations. The additional costs are justifiable by the value added from information gained via genetic analyses. Nonetheless, adoption of new protocols can be a

slow process, particularly when there is disagreement about the rationale and outcomes of use. Indeed, there is currently no consensus on the best methods for aquatic environmental assessment in Hawaii. Methods favored by the US EPA that rate condition according to measures of total species diversity are not favored by state agencies in Hawaii, which instead rely on methods that rate conditions according to population densities of native species. An integrative approach, as outlined here, would help bridge this divide and provide data that would satisfy the objectives of state and federal regulatory agencies. Integrative protocols that are tailored to capture estimates of genetic variation alongside measures of population density and species diversity would be particularly useful for detecting and rating impairment on Oahu. Early use by the DoD might also set a precedent that would encourage subsequent adoption by other institutions.

Integrative assessment approaches and novel perspectives on managing oceanic island stream ecosystems are more likely to be embraced if additional research were to be carried out to resolve some key remaining questions and uncertainties. There are three areas that warrant further study: (1) control and mitigation of aquatic invasive species; (2) drivers of life history variation; and (3) the influence of climate change on water availability and population connectivity in at-risk native species.

Control and mitigation of aquatic invasive species. Aquatic invasive species (AIS) are a widely recognized threat to native and endemic species (Brasher 2003), yet no consistent effort has been made to develop protocols for management in oceanic island streams. As a result, AIS are rampant and poorly understood on Pacific islands (Englund and Filbert 1999). Our findings indicate that non-native fishes (e.g., Poeciliids) constrain native fish densities by limiting recruitment of juveniles, likely through a combination of predation and competition. These results serve to underscore the importance of understanding the mechanisms by which AIS influence native species and suggest that greater benefit could be derived from AIS management than more costly habitat restoration projects.

Management of AIS in Pacific Island stream ecosystems is particularly challenging because traditional methods for control or mitigation do not account for characteristics of insular streams, and societal sensitivities associated with water resources and threatened or endangered species management. Thus relatively little is known about the feasibility or potential outcomes of AIS removal in Pacific Island streams, and even less is known about mitigative watershed management approaches, such as deliberate regulation of stream hydrology (Englund and Filbert 1999, Kiernan et al. 2012), to sustain native biota in the presence of AIS. Development of actionable information and innovative approaches for managing AIS therefore could substantively improve stewardship of stream ecosystems that cross DoD lands, especially on islands where installations harbor native species under federal or state protection.

Drivers of life history variation. Successful conservation actions rely on an adequate understanding of the biology of the focal species, especially when management efforts involve contentious interventions. Preserving ocean-stream connectivity and remediating downstream habitats (i.e., at or near the stream mouth) would likely provide great benefits to species that are obligately amphidromous (Walter et al. 2012), with the expectation that local populations would be open to immigration, and declining populations could rely on rescue from other proximate or distant sources. Conservation of native stream fauna in the Hawaiian Islands has largely followed

this model, despite some indication (Hodges and Allendorf 1998) that long-distance dispersal likely has little influence on local populations of at-risk species. Our analyses of otolith microchemistry indicate that a large proportion of *A. stamineus* larvae remain in natal freshwater or local near-shore environments rather than undergoing marine dispersal, suggesting that local processes likely play a much larger role in population persistence. Thus, populations would likely benefit most from improving in-stream conditions such as habitat alteration, flow regimes and invasive species (Brasher 2003, Walter et al. 2012). However, at present, we do not know whether the observed patterns of variation in self-recruitment, retention, and dispersal are temporally stable. It is also unclear whether life-history variation is heritable or if it is a plastic response to prevailing environmental conditions such as stream flow or the presence of AIS. Conditions in oceanic island streams (in Hawaii, and in general) can be highly variable, potentially creating a temporally dynamic balance of self-recruitment and condition-dependent dispersal, where in some years marine-mediated dispersal is favored but otherwise non-amphidromy wins the day. Further studies will be necessary to determine the full range and drivers of life history flexibility in *A. stamineus* and other amphidromous species native to the Hawaii Islands and other areas of interest in the Pacific.

Climate change, water availability and population connectivity of at-risk native species. Climate change is expected to present significant challenges to military readiness and stewardship requirements across DoD assets on Pacific islands. Freshwater resources are of particular concern because climate-driven reductions in water availability and increasing demand for water could exacerbate existing stresses and give rise to novel stresses on at-risk native species. Thus, DoD management of water resources on Pacific islands must balance mission-critical use against minimum in-stream flow conditions to maintain at-risk native species.

Setting appropriate regional and installation-specific water resource management objectives requires better understanding of how shifts in water availability will influence the vulnerability (exposure, sensitivity, and adaptive capacity) of at-risk species. Amphidromous fish and invertebrates, the dominant native organisms in Pacific island freshwater ecosystems, are especially at risk. Interruption of ocean-stream connectivity by climate change or other factors (e.g., diversions) can eliminate migratory pathways for emigrating larvae and returning postlarvae, and potentially intensify exposure of all life stages to predators and contaminants. As a result, local populations or even entire species could be lost from Pacific island streams. Greater demand for surface water is almost certain if climate-driven declines in groundwater recharge reduce sustainable withdrawal rates (Giambelluca et al. 1986), yet the adoption of interim and permanent in-stream flow standards on Pacific islands has been contentious because little is known about flow characteristics necessary to allow export of larvae and re-entry of postlarvae to sustain resident populations of at-risk amphidromous species. Even the effects of stream flow reductions and intermittent loss of ocean-stream connectivity through temporary stream drying are poorly understood. Therefore a critical step forward in setting appropriate management objectives, including adoption of in-stream flow standards, will be determining the hydrological conditions necessary to enable dispersal, reproduction and survival of at-risk species under current and future climate conditions.

Improved methods for evaluating potential outcomes of alternative adaptive or mitigative management strategies are also needed. Predictive models that couple information on climate, hydrology and demography would enable DoD managers to establish and maintain best

management practices. Spatially explicit models might indicate, for example, that “business as usual” is an acceptable management plan if oceanic dispersal affords amphidromous species the capacity to track available habitat among watersheds and islands. Model-based decision tools could also help minimize costs and maximize benefits by balancing trade-offs of regulating water use, constraining land use practices, or targeted control of invasive species. For instance, a modeled outcome might indicate that scheduling water use to protect sensitive life history stages of native species could be done at little cost. If regulating water use is too disruptive, removing predatory invasive species could be a cost-effective alternative for sustaining at-risk populations. Evaluating cost and benefit functions to determine where return on investments would be greatest under different stream condition, flow and climate scenarios could provide DoD managers objective, defensible guidance for sustaining water availability and conserving at-risk native species.

In summary, the research presented here has expanded fundamental knowledge of insular stream ecology and responses of native amphidromous fishes to environmental stressors. The studies have clarified the extent to which core biological measures of stream condition reflect local degradation and the loss of connectivity between streams and adjoining near-shore habitats. Not only has this work generated a conceptual framework and hands-on tools for implementing integrative monitoring and assessment of oceanic island stream conditions, it has yielded practical recommendations for how DoD resource managers should consider archipelago-scale processes in relation to local conditions when setting management and restoration priorities. With an expanded knowledge base and toolkit, DoD resource managers and partner organizations should therefore be able to achieve more effective watershed management and conservation of at-risk aquatic species to ensure the sustainability of military operations in the Hawaiian Islands and other oceanic islands in the Pacific.

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8 Appendix A. Scientific and Technical Publications

In print, in revision, or in review:

Gagne, R., Blum, M.J., Hogan, J.D, Pracheil, B.,Hain, E.F., McIntyre, P.B., Gilliam, J.F. Infection of a native amphidromous fish by a non-native parasite in Hawaii. *Diversity and Distributions* (in review)

Hansen, G.J.A., Vander Zanden, M.J., Blum, M.J. Clayton, M., Hain, E.F., Hauxwell, J., Izzo, M., Kornis, M.S., McIntyre, P.B., Mikulyuk, A., Nilsson, E., Olden, J.D., Papeş, M., Sharma, S. 2013. Are invasive species more abundant than native species? *PLoS One* Online early. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0077415

Hogan, J.D., Blum, M.J., Walter, R.P. 2010. Characterization of ten novel microsatellite markers in *Awaous guamensis* with comments on cross amplification in congeners and other amphidromous fish native to Hawaii. *Conservation Genetics Resources* 3:275-277.

Hogan, J.D., Bickford, N., Gilliam, J.F., Blum, M.J., McIntyre, P.B. 2014. Consequences of alternative dispersal strategies in a putatively amphidromous fish. *Ecology* (in press)

Lindstrom, D.L., Blum, M.J., Walter, R.P., Gagne, R., Gilliam, J.F. 2012. Molecular and morphological evidence of distinct evolutionary lineages of *Awaous guamensis* in Hawaii and Guam. *Copeia* 2:294-301

McIntyre, P.B., Reidy Liermann, C., Childress, E., Hamann, E.J., Hogan, J.D., Januchowski-Hartley, S.R., Koning, A.A., Neeson, T.M., Oele, D.L., Pracheil, B.M. 2014. Conservation of migratory fishes in freshwater ecosystems. In Closs, G., Krkosek, M., Olden, J.D. eds. Conservation of Freshwater Fishes (in press).

Moody, K.N., Hunter, S.N., Childress, M.J., Blob, R.W., Schoenfuss, H.L., Blum, M.J., Ptacek, M.B. Local adaptation despite high gene flow in the waterfall climbing Hawaiian goby, *Sicyopterus stimpsoni*. *Molecular Ecology* (in review)

Walter, R.P., Blum, M.J., Hogan, J.D, Gagne, R., McIntyre, P.B., Gilliam, J.F. 2012. Climate change and conservation of native fishes in Hawaii. *Endangered Species Research* 16:261-272.

In preparation:

Blum, M.J. et al. Genetic tools for assessing environmental impairment. For *Bioscience.*

Blum, M.J. et al. Comparative phylogeography of amphidromous fauna and contemporary connectivity across the Hawaiian archipelago. For *Molecular Ecology*.

Blum, M.J. et al. Integrative assessment of stream condition across the Hawaiian archipelago. For *Environmental Science & Technology*.

Childress, E. et al. Use of stable isotope assays for assessing water quality and watershed land use in Hawai'i. For *Ecological Indicators.*

Darrah, T, et al. A trace element map of the Hawaiian archipelago based on stream chemistry. For *Applied Geochemistry.*

Gagne, R. et al. Temporal stability of introduced parasite infections of native hosts. For *Biological Invasions.*

Gagne, R. et al. Incongruent genetic structure of introduced parasites and native hosts. For *Evolutionary Applications.*

Gagne, R. et al. Infection of a native host species by a non-native parasite across a climatic gradient. For *Global Change Biology.*

Hain, E.F., et al. Biological gauntlets limit adult densities of migratory fish. For *Ecological Applications.*

Hain, E.F., et al. Demographic and genetic evidence of recruitment limitation induced by exotic species. For *Molecular Ecology*.

Hogan, J.D. et al. $\delta^{18}O$ provides fine-scale resolution of marine dispersal of diadromous fishes. For *Biology Letters.*

Hogan, J.D. et al. Fine-scale spatial and temporal genetic structure of an endemic diadromous fish across its entire range. For *Molecular Ecology*.

Hogan, J.D. et al. Early life-history of two endemic Hawaiian amphidromous fishes. For *Freshwater Biology*.

Kraemer, B, et al. Watershed land use and substrate age as controls on carbon and nutrient stoichiometry in Hawaiian streams. For *Limnology and Oceanography.*

Lamphere, B.A.., et al. Snorkel surveys and mark-recapture methods for assessing amphidromous fish populations in Hawaiian streams For *Fish and Fisheries.*

Lamphere, B.A., et al. Microhabitat use of native and introduced Hawaiian stream fish. For *Pacific Science.*

Lamphere, B.A., et al. Local versus regional drivers of demography and individual growth in a migratory stream fish as inferred from mark-recapture studies and otolith microchemistry. For *Ecology.*

McIntyre P.B. et al. Reciprocal effects of nutrients and species invasions during human transformation of Hawaiian streams. For *Ecosystems.*

Moody, K. et al. Biophysical modeling of ocean-stream connectivity across the Hawaiian archipelago. For *Limnology and Oceanography.*